# The Cognitive Effects of Acute Administration Of Herbal Remedies to Healthy Volunteers.

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Thesis submitted for the qualification of PhD to the University of Northumbria, Newcastle-Upon-Tyne.

The research described within this thesis was undertaken in the Department of Psychology, Faculty of Social Sciences, University of Northumbria.

Submitted April 2002

## Abstract

A number of Oriental and European herbal remedies have been historically attributed with cognition enhancing properties. They also exhibit physiological effects that may be commensurate with the enhancement of cognitive performance. This thesis examined the cognitive and mood effects of acute administration of *Gingko biloba, Panax ginseng,* a combination of ginkgo/ginseng, *S. lavandulaefolia* (Sage) and *Melissa officinalis* (Lemon balm) to healthy volunteers, in a series of double-blind, placebo-controlled, balanced cross-over, multiple-dose, multiple time-point experiments. In the case of each herbal remedy, treatment related changes in cognitive performance were quantified with computerised assessment tools (the CDR battery and serial subtraction tasks), and mood was assessed with Bond-Lader visual analogue scales. Relevant *in vitro* properties of both sage and lemon balm were also assessed. In separate studies electroencephalograph (EEG) recordings following both *Ginkgo biloba* and *Panax ginseng,* and blood glucose levels obtained following the latter of these, were also analysed. The results show that all of the herbal remedies under investigation are capable of modulating cognitive performance, mood, and, where they have been assessed, physiological parameters.

The most striking results include: improved speed of attentional tasks following ginkgo; improved serial subtraction performance following both ginkgo and the ginkgo/ginseng combination; consistently improved secondary memory performance following ginseng and the ginkgo/ginseng combination, but with decrements in the speed of performing attentional tasks for the less optimum doses of each; modulation of EEG recordings following both ginkgo and ginseng; improved memory performance and mood following sage; and decrements on timed memory tasks for lemon balm, but with differential modulation of mood and improved accuracy of memory task performance depending on the dose and cholinergic properties of the specific treatment. The majority of these studies represent either the first studies to investigate cognitive modulation in humans, or alternatively the first studies to investigate the cognitive effects of single doses in humans, of each of the treatments under investigation.

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Acknowledgements

My thanks go to the following for their assistance:

Dr Andrew Scholey and Professor Keith Wesnes for their support as supervisors.

Pharmaton SA (Lugano) for financial and practical support for this PhD programme, and in particular Dr Orlando Petrini and Sheila Campbell for their advice and assistance.

Cognitive Drug Research (CDR Ltd) for provision of their assessment battery and hardware.

Lesley Drewery and Robert Steel for technical assistance whenever required.

Nicola Tildesley for her collaboration on the *Salvia lavandulaefolia* study.

Professor Elaine Perry and Professor Heather Ashton for their advice and assistance on the European herbs section and EEG studies respectively.

Dr George Wake for undertaking the *in vitro* analyses reported in chapters 9, 10 and 11.

Richard Marsh for his technical assistance on the EEG study.

Kirsty Black for patience beyond the call of duty.

## Author's declarations

This work has not been submitted for any other award. It is solely the work of the author.

# CHAPTER 1. INTRODUCTION

## 1.1. General Introduction

Evidence presented to the House of Lords Select Committee on Science and Technology (6th Report, 'Complementary and Alternative Medicine', 2000) suggests that herbal products make up a significant proportion of the total consumption of medicines and other health products in the United Kingdom. The committee heard that in line with research from the USA, a handful of herbal products – Ginkgo biloba, St John's Wort, Ginseng, Garlic, Echinacea, Saw Palmetto and Kava Kava – constitute the lion's share of the herbal medicine market.

Total annual sales in the UK herbal products sector have been estimated to amount to somewhere between £93 million (for the retail sector alone) and £240 million per annum in 1998 (including all forms of retailing) (House of Lords Select Committee on Science and Technology, 6th Report, 2000). However, the results of a telephone survey of the complementary medicine usage of 1204 randomly selected British adults (Ernst and White, 2000) suggest that herbal medicines constituted 34% of the instances of complementary and alternative medicine usage, in a market that the authors extrapolate to be worth, in its entirety, something in the region of £1.6 billion per year. This suggests that the figure for herbal product sales could be somewhat higher than previously estimated.

A similar study in the USA showed that between 1990 and 1997 the use of herbal products increased nearly five fold, from 2.5% to 12.1% of all instances of complementary and alternative medical treatment. This reflects the second highest % usage (after relaxation techniques) amongst the estimated 42.1% of the USA population using alternative treatments. The complementary and alternative medicine industry in the USA is estimated to have a combined turnover of $27 billion annually (Eisenberg *et al,* 1998), and direct estimates of the size and continuing expansion of the US herbal market in 1997 varied between $1.6 billion with a growth of 7 to 10 percent a year, and $3.24 billion, with expansion somewhere in the region of 20% per year (Scimone and Scimone, 1998). This expansion is reflected in estimates of the size of the USA herbals market, which suggest that in 2000 sales were in the region of $4

billion (Ernst, 2000). Whatever the total size of the market, a sizeable and increasing proportion of the US population regularly uses herbal medicines, with this figure most recently estimated at 14% of the adult population (Kaufman *et al,* 2002).

These figures also have to be seen within the context of worldwide herbal retail sales (1997) of $16.5 billion, with Europe accounting for 45 percent of the total sales (Scimone, 1997; Scimone and Scimone, 1998). The phytopharmaceutical market in Germany alone was estimated in 1996 to be worth $3 billion annually (Brown, 1996).

Whilst these figures are impressive, what is more germane to the current thesis is the level of consumption of two of the most popular herbal products: *Ginkgo biloba,* and ginseng, both of which occupy permanent positions in the top selling handful of herbal products, and which have combined sales globally running into billions of dollars (e.g. Croom and Walker, 1995; Rawls, 1996; Springen and Crowley, 1997). What is particularly relevant is that both herbal products are purchased by consumers who believe not only that they will engender physical benefits, but also that they will have a positive effect on their cognitive performance and well-being. As an example of this, the Hartman Group's Natural Products Census Supplement Report (July 1998 - July 1999), shows that *Ginkgo biloba* and ginseng are the first and third (Vitamin E being second) most frequently taken products for 'memory loss' and 'absentmindedness'. This figure is derived from 116.3 million surveyed incidents of herbal medicine usage in the USA.

The question that this thesis will address will be whether herbal remedies have any beneficial effects on cognitive performance. The studies that make up this thesis will include investigations of the cognition enhancing properties not only of the widely taken Oriental herbal extracts *Ginkgo biloba* and *Panax ginseng,* but also the first scientific investigations in humans of the effects of European plants traditionally used to improve mental abilities. Here the focus will be on two members of the Labiatae family; *Melissa officinalis* and *Salvia officinalis,* for which converging lines of evidence suggest the possibility of a role in cognition enhancement.

The remainder of this introduction includes a review of the evidence, across a number of disciplines, pertaining to *Ginkgo biloba* and ginseng (both alone and in a combination product), *Melissa officinalis,* and *Salvia officinalis* and its close relative *Salvia lavandulaefolia.* Also included is a brief review of the evidence of efficacy of a number of plant extracts, and combinations of extracts, mainly drawn from Ayurvedic, Chinese, and Japanese traditional medicine, which are not investigated in the experimental section of this thesis, but which may owe demonstrations of cognition enhancing properties to similar mechanisms as the above.

## 1.2 Ginkgo biloba

### 1.2.1 General Information

The *Ginkgo biloba* tree is the sole member of the ginkgo genus (Clostre, 1999), and is one of the oldest surviving tree species on earth (Major, 1967). Fossil evidence suggests that it has been in existence, largely in its current form, for up to 200 million years, and it benefits from a life span of up to a thousand years (Foster, 1996). Extracts and infusions made from its leaves have been used in traditional Chinese medicine for thousands of years. Currently it is sold 'across the counter' throughout the western world, and ranks as the best selling herbal medication in a market place worth approximately $4 billion in the USA alone (Ernst, 2000; Jacobs and Browner, 1999). In European countries, most notably France and Germany, its prescribed therapeutic use has also grown dramatically following the development in the mid 1960's of manufacturing processes capable of producing standardised extracts. These extracts, derived by a complex drying process, are concentrated in a ratio of approximately 1 part extract to 50 part dried leaves, and are generally standardised to a content of 24% - 25% flavonoids and 6% terpenoids (e.g. GK501, Egb761, LI 1370). Because of the many active ingredients present in ginkgo extracts it is difficult to assess the specific pharmacological mechanisms underlying its effects, but synergistic, additive and antagonistic interactions are likely due to the wide range of active compounds and multiple physical sites of activity.

The most important active substances are likely to be the flavonoids (ginkgo-flavone glycosides of kaempferol, quercitin, and isohamnetin) and the terpenoids (bilobalide and ginkgolides A,B,C and J) (Kleijnen and Knipschild, 1992).

***Pharmacokinetics***

A number of studies have examined the pharmacokinetic properties of the constituents of ginkgo. Fourtillan *et al,* (1995) reported, in 12 healthy young volunteers, bioavailability coefficients of 0.8, 0.88, and 0.79, and mean elimination half lives of 4.5, 10.57 and 3.21 hours

for ginkgolides A and B and bilobalide respectively. It had also previously been demonstrated (Nieder, 1991), using 2 healthy volunteers, that flavonol glycosides were absorbed in the small intestine, had a half life of between 2 and 4 hours, reached peak plasma concentrations within 2-3 hours, and were totally eliminated within 24 hours.

Utilising rats fed a radioactive ginkgo extract, Moreau *et al,* (1986) found that radioactivity during the first 3 hours was primarily associated with the plasma, but through a gradual uptake the specific activity in erythrocytes eventually matched that of plasma. Glandular and neuronal tissues (in particular the hippocampus, corpus striatum and hypothalamus), and the eyes showed a high affinity for the labelled substance.

In humans, following ginkgo extract consumption, metabolites of flavonoids accounting in total for less than 30% of flavonoids consumed, had been detected in collected urine, but not in the blood, after 3 days (Pietta *et al,* 1997). The authors of this study also noted metabolite results suggesting that a more extensive metabolism of ginkgo takes place in humans than in rats.

The 'bio-availability' of ginkgo has been confirmed by the results of a number of EEG studies. For example, (Luthinger *et al,* 1995) demonstrated treatment related EEG effects at testing sessions ranging from 1 to 6 hours post-dose, in healthy young volunteers administered 80 mg and 160 mg of Egb 761.

***Toxicology and Adverse side effects***

A number of research laboratory reports, reviewed by Clostre (1999), suggest that *Ginkgo biloba* (Egb 761) has very low toxicity. Findings include an $LD_{50}$ for oral consumption of 7.7g/kg in the mouse and greater than 10g/kg in the rat. Chronic dose studies over six months have also shown no adverse effects for up to 500mg/kg/day in the rat, and effects restricted to facial reddening for the highest doses in dogs treated with up to 400 mg/kg/day. Similarly Clostre (1999) notes that there is no evidence of embryotoxicity, mutagenicity, or carcinogenicity.

Whilst ginkgo is associated with the possibility, in rare cases, of mild gastro-intestinal upsets, head aches and skin allergies, no serious side effects have been reported in trials involving humans. Where mild effects have occurred, levels have been matched or exceeded by placebo groups (Kleijnen and Knipschild, 1992). The picture is similar with regard to prescribed medical usage. Clostre (1999) reviews a number of reports showing, for instance, that of over 3 million patients treated with *Ginkgo biloba* extract between 1975 and 1985 in France, only 113 adverse side effects were reported. He also notes similar figures for Germany. Whilst ginkgo is generally considered safe, several cases of potential treatment related haemorrhaging have been reported, and interaction with other medications, particularly anti-coagulants, are possible (Fugh-Berman, 2000).

### 1.2.2 Mechanisms of Action

Evidence suggests that *Ginkgo biloba* has a number of physiological properties. It most notably antagonises platelet activating factor, has anti-oxidant and free radical scavenging properties, and can modulate neurotransmission. These may result in observed effects that include modulation of cerebral and peripheral blood flow and cellular metabolism, and a neuroprotective role in the face of a number of deleterious events.

***Antagonism of platelet activating factor***

The ginkgolides, in particular ginkgolide B, are specific platelet activating factor (PAF) antagonists (Smith *et al,* 1996). A potent inflammatory autacoid, PAF not only induces platelet aggregation but also plays an important role in a number of neurotransmission and cellular events (Logani *et al,* 2000). It has been suggested that PAF is implicated in a number of pathologies including asthma, shock, ischaemia, renal disease, central nervous system disorders, and numerous inflammatory conditions. It is implicated in hypoxic damage in various models of cerebral ischaemia (Braquet and Hosford, 1991; Logani *et al,* 2000).

***Free Radical Scavenging***

Free radicals play a role in the general processes of ageing and tissue damage. Oxidative stress is implicated in the evolution of many degenerative diseases including those involved with the nervous system, cardiovascular system, eye dysfunction and carcinogen metabolism (Droy-Lefaix, 1997). It is also suggested to be both a cause of, and caused by, the physiological changes implicit in Alzheimer's disease (e.g. Blass, 1993; Christen, 2000; Frolich and Reiderer, 1995; Markesby, 1997; Ramassamy *et al,* 1999).

*Ginkgo biloba* has been shown to protect post-mortem human brain tissue from free radical induced oxidative damage (Siddique et al, 2000), inhibit free radicals (Hibatallah *et al,* 1999) and to specifically scavenge the superoxide anion, hydroxide radicals, peroxyl radicals (Droy-Lefaix, 1997) and nitric oxide (Marcocci *et al,* 1994). Free radical scavenging is a possible mechanism in many of the physiological effects of ginkgo outlined below. For instance, the free radical scavenging role is implicated both in the prevention of oxidative stress in mitochondria during hypoxia, and in the prevention of mitochondrial DNA damage associated with ageing in rats (Droy-Lefaix, 1997). It plays a possible role, via protection of membrane structures, in ginkgo's effects on neurotransmitter uptake (Ramassamy *et al,* 1992), protects against cerebral ischemic damage (Spinnewyn *et al,* 1986), preserves cells from arterio-sclerotic evolution and protects against reperfusion in the post-ischemic heart (Droy-Lefaix, 1997).

***Neurotransmission***

It has been suggested that *Ginkgo biloba* modulates a number of neurotransmitter systems with, for instance, inhibition of MAO (Kristoikova and Klaschka, 1997), increases in synaptosomal uptake of 5-HT (Ramassamy *et al,* 1992a), and reduction in glucocorticoid synthesis (Amri *et al,* 1996). Evidence also suggests that ginkgo specifically interacts with the cholinergic system. As an example ginkgo has been shown to attenuate scopolamine induced amnesia in rodents (Chen *et al,* 1991; Chopin and Briley, 1992), up-regulate hippocampal muscarinic receptor populations (Taylor, 1986), elevate high affinity choline uptake (Kristoikova and Klaschka,

1997), and indirectly modulate cholinergic function through regulation of the serotonergic system (Nathan, 2000).

Several studies utilising rats have suggested a role for ginkgo in the attenuation of age related changes in post-synaptic receptors with, for instance, increased binding to 5-HT receptors in the cerebral cortex membranes (Huguet *et al,* 1994), increases in muscarinic receptor populations in the hippocampus of aged rats (Taylor, 1986), and increases in binding to alpha 2-adrenoreceptors in the hippocampus of old but not young rats (Huguet and Tarrade, 1992).

***Effects on blood circulation***

Ginkgo has been shown to exert a beneficial effect on blood circulation both through effects on the vaso-regulating activity of arteries, veins and capillaries, and on haemorrheological properties.

As an example, Topp *et al* (2001), using *in vivo* microscopy, demonstrated improved hepatic microcirculation and reduced leukocyte adherence following ischemia in a sample of rats treated with Egb761 20 minutes prior to reperfusion. In conscious rats ginkgo extract has been shown to significantly increase local cerebral blood flow by as much as 100% in 36 of the 39 anatomically defined brain structures analysed (Kriegelstein *et al,* 1986).

In healthy adults Chung *et al* (1999) demonstrated improvements in ocular blood flow following 2 days administration of 120 mg of *Ginkgo biloba* extract. Similarly, a randomised, placebo controlled, single-blind cross over study (Jung *et al,* 1990) involving 10 participants ingesting a single dose of ginkgo and an identical placebo, demonstrated a marked increase in blood flow in the nail fold capillaries, with a mean increase in erythrocyte velocity of 57%. Fluidity of the blood was also significantly improved with an average decrease in erythrocyte aggregation of 15.6%. A number of other haemorrheological parameters were unchanged.

Benefits in both central and peripheral perfusion have also been demonstrated in young, healthy climbers in whom treatment with 2 doses of 80 mg Egb761 per day, as opposed to placebo, reduced not only cerebral and respiratory symptoms of altitude sickness, but also both

subjective reports and plethysmograph measurements of vasomotor disorders of the extremities (Roncin *et al,* 1996).

A number of studies have also investigated the haemorrheological effects of ginkgo in pathological populations. For instance, Koltringer *et al* (1993) demonstrated significant dose-dependent increases in microcirculation, and increases in visco-elasticity, in 42 patients with pathological visco-elasticity values, treated with a single intravenous injection of 50, 100, 150 or 200 mg of Egb 761. Similarly, Witte *et al* (1992) demonstrated an improvement in elevated fibrinogen levels and improved haemorrheological properties in an open study involving 20 patients treated with 240 mg/day Egb 761 for 12 weeks.

A number of studies suggest that ginkgo improves peripheral perfusion in cases of intermittent claudication or peripheral arterial occlusive disease (Ernst 1996), with, for instance, demonstrations of significant improvements in both pain free and maximum walking distances in comparison to placebo controls (Peters *et al,* 1998; Pittler and Ernst, 2000), and in comparing a higher dose (240 mg), to a lower dose (120 mg) of Egb 761 (Schweizer and Hautmann, 1999).

***Changes in Metabolism***

Ginkgo extract has been shown to reduce cortical glucose levels (Kriegelstein *et al,* 1986) and to elicit slight reductions in glucose utilisation in several brain areas under normal conditions in the rat (DuVerger *et al,* 1995). *In vitro* experiments suggest a direct effect of ginkgo extract on cellular glucose transport and utilisation (Bruel *et al,* 1989), and a protective effect of ginkgo under hypoxic conditions. For example, a suppression of hypoxia induced membrane breakdown in brain tissue by bilobalide (Klein *et al,* 1997), and protection of endothelial cell ultrastructure (Welt *et al,* 1996) and mitochondria (Fitzl *et al,* 1996) against hypoxic alterations.

Both bilobalide and Egb 761 have also been shown to inhibit the hypoxia induced decrease in ATP in endothelial cells and delay the onset of glycolysis activation, putatively by protection of

mitochondrial respiratory activity (Janssens *et al,* 1999). It is suggested that this delay in the cascade of cellular events, leading ultimately to the release of inflammatory mediators during hypoxia, accounts for ginkgo's neuroprotective properties following ischaemia (Janssens *et al* 1995). A number of *in vivo* experiments have also demonstrated a hypoxia protective effect of ginkgo extract. For example, using rats, Chen *et al* (1996) demonstrated the relief of hypoxic pulmonary hypertension, whilst Rapin *et al* (1986) found a partial reestablishment of glucose consumption and an increase in glucose transfer rate during hypoxia. Oberpichler *et al* (1988) demonstrated prolonged survival time under lethal hypoxia in mice, and retardation of the breakdown of brain energy metabolism, and increased local cerebral blood flow, in 35 brain areas of hypoxic rats administered both ginkgo extract and a non-flavone fraction of ginkgo.

In humans, a randomised, placebo-controlled, double-blind, cross-over study, involving 8 healthy male adults, demonstrated improvements in respiration rate, corneo-retinal resting potential of the eye, saccadic eye movements, and complex choice reaction times during hypoxic-hypoxia, following 14 days treatment with ginkgo extract (Schaffler and Reeh, 1985). A less direct study by Roncin *et al* (1996) demonstrated the attenuation of the symptoms of mountain sickness in those of 44 mountaineers who took Egb 761 in comparison to those who took a placebo.

***Neuroprotection***

Evidence suggests that *Ginkgo biloba* has a number of neuro-protective properties. *In vitro* it has been shown to protect against a variety of neuro-toxic insults, e.g. the dopamine neuro-toxicity of MPTP (Ramassamy *et al,* 92b), and an ascorbic acid induced decrease in synaptosomal membrane fluidity (Ramassamy *et al,* 1993). It has also been shown to inhibit apoptosis following both serum deprivation and staurosporin treatment of rat hippocampal neurons (Ahlmeyer *et al,* 1999). Similarly, Xin *et al* (2000) demonstrated protection of cerebellar granule cells from apoptosis induced by hydroxyl radicals, following pre-treatment

with both a whole extract (Egb761) and the flavonoid (but not terpenoid) fraction of *Ginkgo biloba*.

Whilst the aetiology of Alzheimer's disease is as yet un-delineated, increased levels of oxidative damage in the frontal cortex of sufferers of the disease have been reported (Markesbery, 1997). A correlation between both lipid oxidation and anti-oxidant levels in this brain area, and possession of none, 1 or 2 of the ε4 alleles that are implicated in Alzheimer's disease has been established (Ramassamy *et al,* 1999). This last study also demonstrated that *Ginkgo biloba* extracts protect *in vitro* against oxidative damage in frontal cortex tissue from ε3|ε3 and ε3|ε4, but not ε4|ε4 Alzheimer's cases. Interestingly, Yao *et al* (1999; 2001) noted that both the anti-oxidant fractions of *Ginkgo biloba*, and a ginkgo extract devoid of terpenes and flavonoids, failed to protect against β-Amyloid induced cell apoptosis, whereas the whole extract did. They suggest (Yao *et al,* 2001) that the protective effect of the extract, certainly in this instance, is not due to anti-oxidant properties, but rather to another, as yet unidentified, property of the extract.

*In vivo* experimentation has also shown not only that bilobalide facilitates the regeneration of motor nerves following traumatic nerve damage (Bruno *et al,* 1993), but also that it accelerates behavioural recovery in rodents following hemiplegia (Brailowsky and Montiel, 1997), penetrating brain injury (Attella *et al,* 1989) and cerebral ischemia (Tadano *et al,* 1998). Prehn and Krieglstein (1993) also demonstrated reduced ischemia-induced neuronal damage to hippocampal areas following a combination of pre and post-insult administration of Ginkolides A and B to rats. Similarly, pre-treatment of rats with whole extracts of *Ginkgo biloba* (Egb 761) have been shown to improve cerebral blood flow, brain glucose levels, and lactate levels following micro-infarction (Le Poncin Lafitte *et al,* 1980), and to increase ATP levels and prolong life during hypoxia (Karcher *et al,* 1984).

### 1.2.3 Cognitive Effects

#### *1.2.3.1 Animal Studies*

There are a number of studies that have looked at the effects of ginkgo extracts on rodent learning and memory. Findings include: quickened acquisition, improved accuracy and increased frequency of responses on an appetitive operant conditioning task in mice (Winter, 1991); attenuation of scopolamine induced amnesia in a passive avoidance task in rats (Chopin and Briley, 1992); improvement in both short term memory (passive avoidance) and brain membrane fluidity in aged mice (Stoll *et al,* 1996), and facilitation of continuous learning and delayed non-matching to position tasks in aged rats (Winter, 1998).

Of particular interest here are studies showing cognitive enhancement due to ginkgo extracts in young rats. For instance, Petkov *et al* (1993) demonstrated differential dose-dependent improvements in retention of learned behaviour on a number of tasks for both young (3 months) and old (26 months) rats as a result of ingestion of extracts of ginseng, ginkgo and a combination of ginseng and ginkgo (Gincosan®). Rapin *et al* (1994) demonstrated the attenuation of stress induced detrimental changes in both discrimination learning and plasma hormones (epinephrine, norepinephrine and corticosterone) in both young (4 mth) and old (20 mth) rats, with differential patterns of performance improvements between the two groups.

A number of animal studies have also reported an anxiolytic effect of *Ginkgo biloba* with improvements demonstrated in performance on the elevated plus maze (Hasenohrle *et al,* 1996, 1998, Satyan *et al,* 1998), and during sub chronic cold stress (Bolanos-Jiminez *et al,* 1995), social interactions (Chermat *et al,* 1997), and learned helplessness (Porsolt *et al,* 1990).

#### *1.2.3.2 Human studies:*

Research has been directed towards an investigation of the efficacy of ginkgo in the treatment of a number of disorders involving both peripheral and cerebral circulatory disturbances.

*Intermittent Claudication*

A number of studies suggest that ginkgo improves peripheral perfusion in cases of intermittent claudication (Ernst, 1996) with, for instance, demonstrations of significant improvements, in comparison to placebo controls, in both pain free and maximum walking distances (Peters *et al,* 1998) and the cognitive decrements associated with the condition (Draebeck *et al,* 1996).

*Cerebral Insufficiency*

Throughout much of Europe, and in particular in Germany, ginkgo is prescribed for the treatment of a number of ill-defined, age-related complaints which are thought to have a cerebro-vascular aetiology. Kleijnen and Knipschild (1992) review the evidence from 40 controlled trials of ginkgo's efficacy in the treatment of 'cerebral insufficiency', the symptoms of which include: headaches, tinnitus, dizziness, depression, anxiety, tiredness or lack of energy, confusion, and difficulties of concentration and memory. Despite some methodological reservations Kleijnen and Knipschild conclude that all but one of the studies demonstrated significant improvements. Most of these studies, and those reviewed by Hopfenmüller (1994), concentrate on doctors' and patients' evaluations of clinical symptoms. However, a number of studies have made objective measurements of changes in cognitive function. For example, out of the eight studies that met Kleinen and Knipschild's (1992) methodological criteria only one involved objective measurement of cognitive function. In this study Wesnes *et al* (1987) found that a ginkgo group suffering from 'idiopathic cognitive impairment' outperformed a placebo group when combined scores were assessed for both accuracy and reaction times throughout a computerised test battery that assessed the speed and accuracy of information processing, reaction times and memory. Significant improvements in both short term memory and learning rate (Grässel, 1992), and in speed of information processing, in those suffering from 'organic brain syndrome' (Oswald *et al,* 1997) have also been demonstrated in those receiving ginkgo as opposed to placebo.

*Alzheimer's Disease (AD) and Vascular Dementia (VD)*

It has been demonstrated that oxygen free radicals play a role in β-amyloid neurotoxicity (Christen, 2000; Frolich and Reiderer, 1995), that oxidative metabolism is impaired in AD (Blass, 1993), and that there is increased oxidative damage in post-mortem frontal cortex tissue of AD sufferers (Markesby, 1997; Ramassamy *et al,* 1999). Both AD and VD are also characterised by cerebro-vascular impairment. These are two areas that may contribute to the demonstrations of a therapeutic effect for ginkgo.

There is accumulating evidence of ginkgo's utility in the treatment of the psychopathological symptoms and memory impairments associated with Alzheimer's disease and vascular dementia. Oken *et al* (1998) in a review of 57 articles, meta-analysed the 4 studies that satisfied their strict inclusion criteria. They concluded that there was a small but significant effect of treatment with ginkgo on objective measures of cognitive function in AD. Recent investigations include the first US based trial (Le Bars *et al* 1997), comprising data from the 202 patients sufferering from both AD and VD who reached the study's 52 week end point. Results include; a significant improvement from the 0 weeks baseline in the Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-COG) scores for the Egb 761 group, in comparison to a significant deterioration in the scores of the placebo group at 26 weeks; a slight improvement from baseline ADAS-COG scores (0 weeks) for the Egb 761 group, but a continued deterioration in scores for the placebo group at 52 weeks; and a significantly higher score on the Geriatric Evaluation by Relatives Rating Instrument (GERRI) for the Egb 761 group. LeBars *et al* (1997) conclude that ginkgo appears capable of stabilising, and in a substantial number of cases, improving the cognitive performance and social functioning of patients suffering from dementia. In a subsequent 'intent to treat' re-analysis of data from the 309 patients that originally commenced the study (i.e. including drop outs from both treatment groups), Le Bars *et al* (2000) retained the overall pattern of results. This analysis also allowed tentative comparisons with previous trials of both tacrine (Knapp *et al,* 1994), and donepezil

(Rogers *et al,* 1998) in AD sufferers, and Le Bars *et al* concluded that 120 mg of Egb 761 was more potent than the lowest dose of each employed in both trials and only slightly less potent than the highest dose employed. They also noted that a concurrent German trial involving AD and VD cohorts (Kanowski *et al,* 1996) utilised 240 mg Egb 761 and showed comparatively better results, with 38% (as opposed to 26% for Le Bars) of the ginkgo group reaching the highest cut-off point on the cognitive scale. Indeed, Kanowski *et al*'s (1996) study, a 24 week trial involving data from 156 patients, reported improvement 15% in excess of placebo in scores on the Syndrom Kurztest (SKT), which comprises nine sub-tests evaluating attention and memory.

The primary outcome measure of Maurer *et al*'s (1997) study, again utilising treatment of 240 mg/day of Egb 761, was also patients' scores on the SKT. Results of the three month study, involving 20 participants, showed that the patients in the Egb 761 group had improved scores on the SKT, whilst those in the placebo group exhibited deteriorating scores. However, it should also be noted that in sharp contrast to the overall tenor of the above literature, van Dongen *et al* (2000) conducted a somewhat complicated study in elderly patients suffering from mild to moderate dementia and age associated memory decline, comparing the effects of five treatment regimes: two doses of ginkgo (160 and 240 mg/day) for 24 weeks; the same two doses for 12 weeks followed by 12 weeks of placebo; and 24 weeks of placebo. Outcome measures included a clinical assessment, a number of neuropsychological measures, a mood scale, self-reported health and memory, and a behavioural assessment. The most prevalent comparison, an intent-to-treat analysis made between the two combined continuous ginkgo groups (n=79) and the placebo group (n=44), showed no significant differences. Whilst the authors take these results to suggest that, in both of these patient populations, '*treatment with ginkgo is not efficacious, irrespective of dose*' (p1192), it should also be noted both that the majority of patients were suffering from age associated memory decline (70%), and that the size of the individual treatment groups fell below those necessary to reliably detect a clinically significant improvement according to the authors' own power calculations.

*Age associated cognitive decline*

A number of studies that have examined cognitive enhancement in non-pathological human samples have concentrated on the amelioration of age associated declines in memory. For instance, Allain *et al* (1993) showed that a single dose of either 320 mg or 600 mg Egb 761 increased speed of information processing, as measured by a dual coding test, in elderly subjects with slight age related memory impairment.

Israel *et al* (1987), in a placebo controlled study, showed that whereas both a memory training programme and administration of a ginkgo extract improved memory in the elderly (as assessed by the 'Batterie de Mémoire pour Personnes Agées Ambulatoires), a combination of both memory training and ginkgo significantly improved memory beyond the level seen with either treatment alone.

Rai *et al* (1991) examined cognitive functioning in elderly patients showing mild to moderate memory impairment of organic origin. Ginkgo extract (40 mg) was administered three times a day for 24 weeks. Performance on the Digit Copying (but not Object Learning) sub-test of the Kendrick battery was significantly improved at both 12 and 24 weeks, as was reaction time on a computerised classification task. A reduction in the EEG for frequencies between 1 and 3 Hz was also noted and interpreted as possibly reflecting an 'alerting' action of the drug.

*Healthy cohorts*

While evidence is accumulating as to ginkgo's therapeutic efficacy in offsetting declines as a result of age and a number of pathological conditions, there have been also been a number of studies that have addressed this issue in cohorts of older and younger healthy humans. In the former group one recent study (Cockle *et al,* 2000) looked at observer and self-rated measures of activities of daily living, quality of sleep and aspects of mood in a group of 1000 'free-living older volunteers' who received 120 mg ginkgo (LI1370), in comparison to an untreated control group of 4028 participants. Self-rating scales completed at 1, 2, 3 and 4 months following the

commencement of the study showed improvements in all three domains for the ginkgo group in comparison to controls. Whilst the study was not placebo controlled the authors argue that the demonstrated effects were unlikely to be as a result solely of a placebo effect, as the observer ratings of activities of daily living at the commencement of the study and at the 4 month end-point also showed a significant improvement. Similarly, the improvement on the self-rated activities of daily living and sleep scale both showed a pattern of increasing improvement throughout the 4 monthly assessments, a pattern that again would not be in keeping with a straightforward placebo effect.

A more direct investigation of cognitive functioning was provided by Mix and Crews (2000), who reported increased speed on a timed Stroop task (in comparison to placebo), and trends towards increased speed of performance on three further tasks, in a parallel groups investigation of the effects of 6 weeks administration of Egb 761 to 40 'cognitively intact' healthy, older (55-86 years) participants.

Several studies have looked at ginkgo mediated cognitive changes in healthy young participants. As mentioned above, a randomised, placebo controlled, double-blind, cross-over study, involving 8 healthy male adults, demonstrated improvements in complex choice reaction times during hypoxic hypoxia following 14 days treatment with ginkgo extract (Schaffler and Reeh, 1985). However, these results represent a specific protection in deterioration of performance and therefore may have more relevance to studies concentrating on pathological or memory impairment conditions.

The earliest study directly investigating ginkgo's effects in young humans was reported by Hindmarch (1986). This double-blind, cross-over trial involved a small cohort (8) of healthy females. They received three doses of Egb 761 (120 mg, 240 mg, 600 mg) and placebo, counterbalanced over four test sessions at weekly intervals. No significant results were reported with regard to critical flicker fusion and choice reaction time tests, but there was a significant improvement in performance on a short term memory scanning task (Sternberg), with participants exhibiting reduced global response times on the test, with this effect increasing as a

function of the difficulty of the test (i.e. 4, 5 or 6 numbers memorised). This effect was, however, isolated to the 600 mg dose of Egb 761, with the other two doses and placebo generating indistinguishable results. A study by Warot *et al* (1991), involving 12 healthy females, failed to replicate Hindmarch's (1986) findings with the Sternberg test, but did generate an improvement in free recall score for 'Tanakan' (Egb 761) (600 mg) but not for another ginkgo extract product (600 mg) or a placebo. Recently, however, Rigney *et al* (1999) examined the effects of two day administration regimens of four doses (120 to 300 mg) of ginkgo. In a balanced cross-over study, thirty one participants, ranging in age from 30 to 59, were administered a battery of tests at baseline and then at hourly intervals over two days (10 am to 9 pm). In comparison to placebo, performance was only significantly improved on reaction times for the Sternberg numeric working memory task. This effect was seen on days one and two for 120 mg and 300 mg ginkgo extract and on day two alone for 240 mg. These improvements were also more marked for the older participants in the study. Both Hindmarch (1986) and Rigney *et al* (1999) interpreted the improved speed of performing the Sternberg numeric working memory task as reflecting a specific improvement of memory performance, an interpretation that was similarly attributed to the findings of Stough et al (2001), who purported to demonstrate ginkgo related improvements on Digit Span Backwards, and speed on a working memory task, and a delayed auditory verbal learning task, following 30 days administration of ginkgo or placebo to 50 participants. It should, however, be noted that in the case of this last study the findings are difficult to assess as the paper failed to provide adequate details on doses of ginkgo, tasks employed, or results obtained.

Whilst most published studies involving young participants have demonstrated improvements attributed to the treatment on at least one task from within their respective batteries, a recent study by Moulton *et al* (2001) found no interpretable significant differences. The study involved the administration of 120 mg LI1370 or placebo to 60 healthy young males for 5 days in a double blind, between subjects experiment. Tasks included the Sternberg numeric working

memory task, vocabulary and digit span subtests of the WAIS-R, a reading span test, and a prose recall test.

*Electroencephalograph (EEG) studies*

A number of studies have focussed on, or included measures of EEG activity. Findings in impaired populations include, in sufferers from age associated cognitive impairment, shorter P300 wave latency following both acute and chronic administration of 120 mg of a *Ginkgo biloba* extract (Semlitsch *et al,* 1995). In general a high proportion of theta waveband activity in comparison to alpha waveband activity in the eyes closed EEG is thought to indicate disturbed vigilance (Geβner *et al,* 1985). Ginkgo has been shown to 'normalise' these theta/alpha ratios in a number of studies. For instance this effect has been noted during 12 weeks administration of ginkgo to those participants suffering from age associated memory impairment that had an unfavourable initial profile (Gessner *et al,* 1985), and in a cohort of participants recruited on the basis of an unfavourable profile (Pidoux *et al,* 1983). A similar effect has been demonstrated in sufferers from Cerebral Insufficiency following 8 weeks (Hofferberth, 1995; Schulz *et al,* 1991) and 12 weeks (Hofferberth, 1995) administration of *Ginkgo biloba* (in comparison to placebo), and in sufferers from Alzheimer's disease during a three month regime (Hofferberth, 1994). Several studies in the latter patient population also provide some further evidence of beneficial modulation of frequency bands within sub-groups of participants following both acute (Itil *et al,* 1998), and chronic (Kanowski *et al,* 1996; Maurer *et al,* 1997) administration of ginkgo.

Whilst ginkgo's most consistent effect might be said to be a beneficial modulation of disturbed bio-electrical cerebral activity in impaired populations, a number of studies have looked at EEG effects in healthy cohorts. Findings include a dose-dependent increase in alpha waveband activity, in comparison to placebo, in 12 healthy unimpaired adults as a consequence of ingestion of single doses of ginkgo extract (Itil *et al,* 1996). Similarly, Luthinger *et al* (1995) reported increased relative and absolute anterior alpha-1 (8-9.5 Hz) power, with increased

relative anterior alpha-2 (10-12.5 Hz) power, but with decreased absolute power in the same waveband, following single doses of ginkgo. This last study also showed both significant increases and decreases (during 8 testing sessions spanning six hours) in Contingent Negative Variation (CNV) and P300 amplitude. These somewhat mixed results, should also be seen in the light of a previous topographic study (Kunkel, 1993) that showed a large number of treatment-related effects. However, there was no clear pattern to the results, with the three doses (40, 80 and 160 mg Egb 761) and the two fractions of the extract under investigation all generating markedly different profiles of significant resting EEG waveband modulation.

### 1.2.4 Conclusion

A wealth of research suggests that *Ginkgo biloba* extracts and constituents exert a number of effects at a cellular, microscopic, or physiological level that are potentially beneficial in the treatment of cognitive deficits. A good deal of research has been directed toward the question of the role of ginkgo in the attenuation of cognitive deficits due to both pathological conditions and ageing. The lack of negative results in these areas is noteworthy and, even allowing for a publication bias, the current balance of evidence seems to indicate that *Ginkgo biloba* may have a therapeutic role in 'cerebral insufficiency', Alzheimer's disease, and age associated cognitive decline.

However, it must also be noted that the vast majority of the clinical evidence pertaining to *Ginkgo's* efficacy has been obtained with a range of instruments varying from self-report questionnaires to a disparate collection of neuropsychological tasks. Only on rare occasions (e.g. Rai *et al* 1991, Wesnes *et al* 1987) have computerised assessments, with their attendant benefits, been undertaken. Curtis Prior *et al* (1999) sum this up succinctly by suggesting that "*whilst these outcomes are compatible with (but do not affirm) a clinical benefit resulting from the use of Ginkgo, the application of a more objective system of assessment would be able to provide firm proof*" (p540). They go on to advocate the use of computer-based assessment

systems in volunteers and patients, in order to provide the "*convincing evidence currently being sought by patients, carers, physicians, legislators and the pharmaceutical industry*".

It is notable that animal studies suggest beneficial learning and memory effects in young rodents, and that the direct physiological results of ginkgo consumption (EEG, haemorrheological changes, etc...), and concomitant cognitive improvements, can be seen in healthy younger humans. This would tend to suggest that the relative paucity of objective, computer-based evidence might most fruitfully be first addressed in healthy young volunteers.

## 1.3. Ginseng

### 1.3.1. General information

'Ginseng' is generally taken to refer mainly to the dried root of several species in the plant genus *Panax* (Araliaceae family). The most popularly used family member is *Panax ginseng,* which is of Asian derivation (Eastern China and Korea), and has been used for several millennia in Chinese medicine as a tonic, restorative or prophylactic agent (Bahrke and Morgan, 1994; 2000). Other members of the genus include *Panax quinquefolius* (American), *Panax notoginseng* and *Panax japonicus*. Roots of 5 to 7 year old plants are either air dried (white) or steam treated (red).

The major active constituents of ginseng are thought to be triterpenoid glycosides or saponins, also known as ginsenosides, of which over 30 individual examples, many of which only exist in minute amounts, have been identified (Tachikawa *et al* 1999). Ginsenosides can be classified into three groups on the basis of the chemical structure of their sapogenins (aglycones): the panaxadiol group (e.g. $Rb_1$, $Rb_2$, $Rb_3$, Rc, Rd, $Rg_3$, $Rh_2$, $Rs_1$); the panaxatriol group (e.g. Re, Rf, $Rg_1$, $Rg_2$, $Rh_1$); and the Oleanolic acid group (e.g. Ro) (Tachikawa *et al* 1999).

***Standardisation***

The ginsenoside content of ginseng can vary depending on the species, the age and part of the plant, the preservation method, the season of harvest, and the extraction method (Liberti and Der Manderosian, 1978; Phillipson and Anderson 1984). Russo (2001) also notes that no herb is more subject to adulteration and misrepresentation than ginseng, and notes that ginsenoside content in many brands on the US market is low to negligible. This latter point is largely confirmed by Cui et al (1995; 1996) who found a huge variation in ginsenoside content across several dozen commercial ginseng preparations and extracts. Ginseng products have also been found to contain naturally occurring methylxanthines (Vaughan *et al,* 1999), and contaminants such as pseudoephedrine (Bahrke and Morgan, 2000).

The most widely used standardised ginseng extract, both commercially and for research purposes, is G115, a concentrated aqueous extract of *Panax ginseng* contained in the marketed product Ginsana®, which is standardised by rigorous extraction and manufacturing processes to contain an invariable 4% of ginsenosides (Soldati and Sticher 1980).

As a practical example of how poor standardisation might confound research involving ginseng, recent research has shown that ginsenosides from the three groups (panaxadiol, panaxatriol, and Oleanolic acid groups) exert markedly different pharmacological and behavioural effects, with, for instance, differing influences on the *in vitro* responses to various receptor stimuli (e.g. Kudo *et al* 1997; Tachikawa *et al* 1999), and demonstrations of improvements in scopolamine induced learning deficits as a consequence of panaxatriol, but not panaxadiol, administration (Yamaguchi *et al* 1995, 1996). In keeping with this, following results showing a memory enhancing effect in rodents of ginseng extracts with a high, but not low, ratio of panaxatriol/panaxadiol ginsenoside content, Jin *et al* (1999) suggest that the ratio of ginsenosides may be an important factor in the pharmacological effects of ginseng extracts. Indeed, the research outlined below has involved the use of a wide variety of different ginseng products and components, including: standardised extracts; ginseng total saponins (all of the ginsenosides); single or multiple ginsenosides extracted from either the root or the leaves/stem; and different extractions of ginseng (e.g. ether, ethanol, aqueous). By and large the human experimentation reviewed below has involved the use of whole or standardised ginseng extracts.

***Pharmacokinetics***

Possibly due to the lack of satisfactory analytical methods to detect plasma and tissue concentrations there has been little research published on the pharmacokinetics of the absorption, distribution and excretion of ginseng saponins. However Odani *et al* (1983a; 1983b) report that ginsenoside Rg1 was absorbed rapidly from the upper digestive tract, reached peak concentrations in the serum at 30 minutes and in tissue after 90 minutes, and was widely

distributed throughout the body, but was not detected in the brain of rats. Cui *et al* (1996) using gas-chromatography mass spectroscopy also confirmed the uptake of ginsenosides in humans by demonstrating the presence of metabolites in the urine samples of athletes that had consumed ginseng within the last ten days.

### 1.3.2 Possible mechanisms of action

The ginseng literature contains a large number of studies examining a plethora of mechanisms supposedly underlying ginseng's efficacy. Whilst there is a vast amount of evidence that ginseng exerts a number of physiological effects, there is no solid evidence linking any one putative mechanism, or indeed any synergistic system of mechanisms, to behavioural change. This is partly due to the nature of the evidence pertaining to the behavioural or psychological effects of ginseng. Even the animal literature, which provides some evidence of anti-fatigue, anti-stress, and learning and memory effects, is neither consistent nor unequivocal, and, no doubt due to understandable methodological differences, evidence of similar effects in humans is lacking. This makes a thorough discussion of possible mechanisms somewhat redundant, so it may be more appropriate to give a taste of the extant research pertaining to several areas that may be implicated.

***Cardiovascular and Haemorrheological effects***

Intravenous ginseng administration to anaesthetised dogs has been shown to produce a number of effects including a reduction, followed by an increase, in blood pressure, and transient vasodilation (Wood *et al,* 1964). Lee *et al* (1981) administered ether, ethanol and aqueous extracts of ginseng to anaesthetised dogs. Both ether and ethanol extracts decreased total peripheral resistance and caused vasodilation and bradycardia, while the aqueous extract of ginseng led to a significant increase in total peripheral resistance.

Lei and Chiou (1986) found that extract of *Panax notoginseng* decreased systemic blood pressure in rats and rabbits, They suggested that ginseng could be a useful treatment for angina

since it dilates coronary vessels, but that it would not be a useful treatment for hypertension since it can induce both vaso-dilation and vaso-constriction depending on dose and target vessel. Kang *et al* (1995) suggest that ginsenosides induce vaso-relaxation via the release of nitric oxide from endothelial cells, and that this may contribute to the beneficial effect of ginseng on the cardiovascular system. This is, in part, substantiated by the findings of other researchers (Gillis, 1997).

Research suggests that several of ginseng's active ingredients also have a beneficial influence on platelet aggregation. Shi *et al* (1990) demonstrated an anti-atherosclerotic action of total ginsenosides, apparently mediated by a correction in the imbalance between prostacyclin and thromboxane. Kimura *et al* (1988) tested 6 ginsenosides and found that only Rg1 inhibited 5-HT release from, and aggregation of, platelets, which had been induced by adrenaline and thrombin. Kuo *et al* (1990) found that ginsenosides Ro, $Rg_1$, and $Rg_2$ all suppressed the release action of rabbit platelets, but that panaxynol, a non-ginsenoside fraction, inhibited aggregation, release reaction and thromboxane formation. This finding is in agreement with several other studies that have found panaxynol, or the lipophilic fraction, to be the most potent antiplatelet agent in ginseng, chiefly due to an inhibition of thromboxane formation (Teng *et al,* 1989). This possibly occurs via regulation of cGMP and cAMP levels, and prolongation of the time interval between the conversion of fibrinogen to fibrin (Park *et al,* 1996). Ginsenosides have also been shown to be relatively potent platelet activating factor antagonists (Jung *et al,* 1998).

***Cardio-protection and Neuro-protection***

A cardio-protective effect has also been reported, with significantly lower coronary vasoconstriction following hyperbaric hyperoxia in response to angiotensin II, and protection of endothelial function in aortic rings in ginseng treated animals (Gillis 1997). Enhanced recovery of cardiac haemo-dynamic performance and lowered mitochondrial swelling in cardiopulmonary patients has also been shown as a consequence of including ginseng extract in cardioplegic solution during open heart surgery (Zhan *et al,* 1994). It has been suggested that

the cardiovascular protective effects of ginseng may be mediated by the release of nitric oxide (Gillis, 1997; Lim *et al,* 1997).

Neuro-protective properties of ginsenosides have also been demonstrated *in vivo*, with protection of hippocampal CA1 neurons (Chen, 1999; Wen *et al,* 1996), reduction of infarct area (Zhang and Liu, 1996) and preservation of local cerebral glucose utilisation (Choi *et al,* 1996) following ischaemia in rodents.

Possible ginseng mediated neuro-protective mechanisms include: a protection against overproduction of nitric oxide (Kim *et al,* 1998); free radical mediated lipid-peroxidation (Huong *et al,* 1998, Zhang *et al,* 1996); blockade of calcium over-influx into neurons (Liu and Zhang, 1995; Zhong *et al,* 1995); cerebro-vascular relaxation via a nitric oxide pathway (Chen *et al,* 1997) and modulation of cellular energy metabolism (Jiang and Qian, 1995).

***Hypothalamic-Pituitary-Adrenal system regulation***

Some of the 'adaptogenic' effects of ginseng are attributed to its actions on the hypothalamic-pituitary-adrenal system (Sonnenborn and Proppert, 1991). Both situation dependent increases and decreases in corticosterone levels as a consequence of ginseng administration have been demonstrated. Evidence indicates that both oral administration (Filaretov, 1988) and inter-peritoneal injection of ginseng can increase plasma levels of ACTH and corticosterone, with this effect being abolished by hypophysectomy (Hiai *et al,* 1983).

Conversely, ginseng total saponins injected intra-cerebroventricularly have been found to inhibit the stress-induced increase in plasma corticosterone levels as a consequence of the intra-cerebro-ventricular injection in mice. This inhibitory action of ginseng was blocked by a co-administered inhibitor of nitric oxide synthase, suggesting that ginsenosides modulate the stress-induced hypothalamo-pituitary-adrenal response by inducing nitric oxide production in the brain (Kim *et al,* 1998).

Kim *et al* (1998) found that ginseng total saponins and ginsenosides exerted inhibitory effects on Ca2+ currents in rat adrenal chromaffine cells. They suggest that the cellular basis of the

antistress effects of ginseng may be the regulation of catecholamine secretion from adrenal cells.

However it should also be noted that Luo *et al* (1993) demonstrated that whilst coldwater swim stress raised levels of serum corticosterone in rats and mice, both total root saponins and ginsenoside $Rb_1$ inhibited this increase of serum corticosterone in rats. However the same dosage/kg of ginseng/ginsenosides produced an opposite effect in mice, with increases in serum corticosterone levels.

***Modulation of Glucose levels***

It has been demonstrated that ingestion of a number of different types of ginseng, including Asian, American, Korean Red and Canadian white ginseng, can lead to a reduction in fasting blood glucose levels in rodents (Lin and Xiao, 1992; Martinez and Staba, 1984; Ohnishi *et al,* 1996; Oshima *et al,* 1987), and improvement in the glucose tolerance curve of diabetic mice (Oshima *et al,* 1987)

In humans a reduction in fasted blood glucose levels and glycated hemoglobin, in comparison to placebo, has been reported following 8 weeks administration of 200 mg of *Panax ginseng* to 18 participants with Type 2 Diabetes Mellitus (Sotaniemi *et al* 1995). A number of studies have also demonstrated reductions in blood glucose levels following a 25g glucose challenge in both diabetic patients who had ingested 3g, 6g and 9g (Vuksan *et al,* 2000a; 2000b), and non-diabetics administered 1g, 2g and 3g (Vuksan *et al,*2000a, 2001) of *Panax quinquefolius* (American ginseng). Vuksan *et al* (2000a) suggest three possible mechanisms underlying these effects: a ginseng related slowing of the rate of digestion of food (Suzuki *et al,* 1991; Yuan *et al,* 1998); an increase in intra-cellular glucose transport (Hasegawa *et al,* 1994; Ohnishi *et al,* 1996); and modulation of insulin secretion (Kimura *et al,* 1981). It is also noted that the latter two putative mechanisms may well be mediated by increased nitric oxide production (Roy *et al,* 1998; Spinas *et al,* 1998), a phenomenon that has been hypothesised to underlie many of ginseng's physiological effects (Gillis, 1997).

***Modulation of neurotransmission***

Extracts of *Panax ginseng* (Hsieh *et al*, 2000; Jin *et al*, 1999; Nitta *et al*, 1995) and *Panax quinquefolium* (Sloley *et al*, 1999) have been reported to improve the memory deficits associated with scopolamine administration to rodents. The latter of these studies showed increased choline uptake in synaptosomal preparations. It has also been reported (Benishin *et al*, 1991) that ginsenoside $Rb_1$ has a direct effect on acetylcholine metabolism in neural tissue, facilitating the release of hippocampal acetylcholine with an associated increase in the uptake of choline into nerve endings. Benishin (1992) also demonstrated that $Rb_1$ increased the maximum velocity of choline uptake, and that chronic administration of $Rb_1$ increased the number of choline uptake sites in the hippocampus, and to a lesser extent in the cortex. Both ginsenosides $Rg_1$ (Zhang *et al*, 1990) and Rb1 (Salim *et al*, 1997; Zhang *et al*, 1990) have also been shown to increase choline acetyltransferase levels in rodent brains. Possibly more directly pertinent, an *in vitro* investigation of displacement of 3H-(-)nicotine showed that crude extracts of *Panax ginseng* and *Panax quinquefolium* exhibited an affinity for the nicotinic receptor in human brain cerebral cortex membranes. *Panax ginseng* also exhibited an affinity, although to a lesser extent, for the muscarinic receptor (Lewis *et al*, 1999).

Petkov (1978) found that ginseng administration (50mg/kg) led to increases in brainstem dopamine and norepinephrine, and increases in serotonin in the cortex. This action was abolished by administration of either a serotonin receptor agonist or a specific serotonin antagonist, suggesting that serotonergic transmission was involved in the memory enhancing effect. It has also been shown that ginseng total saponin can modulate dopaminergic activity at both presynaptic and postsynaptic dopamine receptors (Kim *et al*, 1998), and can block behavioural sensitisation induced by psychostimulants such as morphine (Kim *et al*, 1995a), cocaine (Kim *et al*, 1995b), methamphetamines (Kim *et al*, 1998) and nicotine (Kim *et al*, 1999; Shim *et al*, 2000). The latter authors suggest that these effects are mediated by the

inhibition of drug related dopamine release by the action of ginseng total saponins on pre-synaptic dopamine terminals.

Wang *et al* (1995) also found that both root and stem/leaf saponins improved learning and raised the levels of biogenic monoamines in normal rats' brains. Ginseng has also been shown to attenuate pentylenetetrazole induced decreases in rat brain monoamine oxidase, possibly accounting for its demonstrated anti-anxiety effect in rodents (Bhattacharya and Mitra, 1991).

***Nitric Oxide synthesis***

As noted above, it has previously been suggested that a number of ginseng's physiological effects are as a consequence of enhanced synthesis of nitric oxide (NO) (Gillis, 1997). Recent evidence of increased NO synthesis throughout the body supports this contention. Examples include demonstrations that ginsenosides play a role in renal protection (Han and Kim, 1996) and cardio protection via enhanced synthesis of NO (Chen, 1996; Maffei Facino *et al*, 1999). The purported immunomodulatory effects of ginseng may also be attributable to increased NO synthesis from macrophages (Fan *et al*, 1995; Friedl *et al*, 2001; Park *et al*, 2001), whilst ginseng related reductions in stress-induced hypothalamo-pituitary-adrenal responses are thought to be mediated by increased NO production in the brain (Kim *et al*, 1998).

It has also been shown that stimulation of NO release underlies ginsenoside mediated relaxation of rabbit (Chen and Lee, 1995; Choi *et al*, 1998; Kim *et al*, 1998) and human (Tamaoki *et al*, 2000) smooth muscle. Whole ginsenoside extracts have similarly been shown to engender *in vitro* nitric oxide dependent vaso-dilation of both primate (Toda *et al*, 2001) and porcine (Chen *et al*, 1997) cerebral arteries, and endothelium-dependent relaxation of rodent aortas (Kang *et al*, 1995; Kim *et al*, 1999). A single study in hypertensive humans also showed increased forearm blood flow following vasodilatory infusions of acteylcholine and bradykinin (but not sodium nitroprusside) in ginseng treated participants, a finding which the authors suggest reflects improvements in vascular endothelial dysfunction as a consequence of enhanced NO synthesis (Sung *et al*, 2000).

Interestingly, ginsenosides have also been shown to inhibit vasodilation via NO dependent inhibitory nerve actions in the rat mesentery (Peng *et al*, 1995).

### 1.3.3 Behavioural and Psychological Effects

#### *1.3.3.1 Ginseng as an 'adaptogen'*

Traditionally, ginseng's role in the Chinese pharmacopeia has been that of a 'tonic', particularly for the elderly and those recovering from illness (Fulder, 1990), a role that is supported by a wealth of anecdotal evidence.

Brekhman and Dardymov (1969) described ginseng as an 'adaptogen'- an innocuous substance which, via non-specific actions, serves to increase resistance to a range of stressors, be they psychological, physical, chemical, or biological. An adaptogen would also serve to normalise or regulate dysregulated functioning. They noted that the effects of ginseng became apparent '*when the resistance of the organism was diminished or the organism was taxed with extra demands*' (p49). In keeping with this, individual ginsenosides have been shown *in vivo* to exert an anti-inflammatory effect (Matsuda *et al,* 1990; 1991), and *in-vitro* to possess anti-mutagenic and DNA protective properties, and to exert a protective action against mammalian tumour cell lines (Ong and Yong, 2000). These and other properties may well underlie demonstrations, from both epidemiological studies and clinical trials, of, for instance, an inverse relationship between frequency and duration of ginseng intake and risk of non-organ specific cancers (Yun and Choi, 1995; 1998), anti-hepatoxicity effects (Zuin *et al,* 1987), and bolstering of the immune system (Scaglione *et al,* 1996). Whilst these and other demonstrations of general health benefits are of undoubted interest, the evidence with regard to 'adaptogenic' effects that may be more germane to aspects of cognitive performance are reviewed below.

*Animal Studies*

Examples of 'adaptogenic' effects of ginseng in animal experiments include demonstrations of stress reduction. For example: the suppression of psychological and foot shock stress induced antinociception in mice (Nguyen *et al,* 1995); an attenuation of the disruption of pentobarbital induced sleep induced by 30 minutes of psychological stress in mice, with no change in sleep duration in unstressed mice (Nguyen *et al,* 1996); protection against psychological stress induced gastric lesions in mice (Huong *et al,* 1998); and an inhibition of intra-cerebro-ventricular injection stress induced increases in plasma corticosterone levels in mice (Kim *et al,* 1998). One of the concomitants of such psychological stress in rodents is an enhancement of lipid peroxidation activity, and Yobimoto *et al* (2000) demonstrated the suppression by Vietnamese ginseng of oxidative damage to brain membranes as a result of a stressful experience (placing of the mouse in a chamber in which it had observed the extended electric shocking of another mouse).

A number of studies have also demonstrated that administration of ginseng or its active components can attenuate fatigue in rodents. The endurance of rodents has been examined on a number of occasions, with ginseng consumption or injection leading to significant increases in endurance time to exhaustion on tests involving swimming (Savel, 1971), treadmill running (Filaretov *et al,* 1988) and continuous rope running (Brekman and Dardymov, 1969). Administration of ginseng has also been shown to be associated with a number of physiological changes. For instance, Filaretov *et al* (1988) found that a single administration of ginseng increased rat endurance times by 32%, and was accompanied by an increase in the basal level of ACTH and corticosteroids, with this effect disappearing by the end of 7 days treatment.

Avakian and Evonuk (1979) demonstrated that administration of 2 mg crude ginseng extract to rats did not affect glycogen levels prior to exercise, but significantly increased glycogen levels after 1.5 hours (39%) and 3 hours (115%) of prolonged swimming. Similarly, Avakian *et al* (1984) demonstrated no effect at rest, but higher levels of blood glucose following 60 minutes

of exercise, and lower concentrations of lactic acid, pyruvic acid and free fatty acid after 30 minutes of exercise for rats treated with ginseng as opposed to placebo.

However, Martinez and Staba (1984) found no increase in endurance times and no effects of ginseng extract on plasma lactic acid, glucagon, insulin, or liver glycogen levels in rested or exercised rats. Similarly, whereas most of the aforementioned studies have not been blinded, Lewis *et al* (1983) utilised a blind design, and found no adaptogenic effects during exercise for four ginseng infusions during a series of trials over three months.

Several recent studies suggest that this variability in results may possibly be attributed either to the quality of the extract of ginseng used and/or to the dose investigated, and a possible habituation either to the effects of ginseng or exercise. For example, Wang and Lee (1998) found that short-term (4 days), but not chronic, treatment with ginseng total saponin significantly prolonged the aerobic endurance of non-trained rats compared to saline treated controls. Ginseng treatment significantly increased the plasma free fatty acid level and maintained plasma glucose level during exercise. They also found that a preparation devoid of ginsenosides $Rg_1$ and $Rb_1$ failed to enhance, whereas injection of either $Rg_1$ or $Rb_1$ enhanced, aerobic exercise performance. Fernando *et al* (1999a) found that both ginseng and treadmill exercise alone improved a number of haematological parameters in rats, but that the combination of ginseng and exercise produced a smaller improvement. These results suggest a clear physiological response due to the ginseng extract administration similar to that obtained after long-term exercise, but no synergistic effect of ginseng and exercise. This possibility was borne out by Fernando *et al* (1999b) who found that prolonged treatment with ginseng (G115) increased the capillary density and the oxidative capacity of rat forelimb muscles, providing greater aerobic potential in a manner similar to the performance of physical exercise. As with the previous research the combination of exercise and treatment failed to potentiate the separately obtained effects.

*Human 'Quality of Life' and 'Well being'*

No research has been conducted into the relief of experimentally induced stress in humans by ginseng. However a number of studies deal with the more generalised question of 'quality of life' or 'well being'.

Neri *et al* (1995) in a double blind, placebo controlled trial involving a cohort suffering age related memory impairment, investigated the effect of a standardised ginseng/vitamin complex on ratings of quality of life, ratings of symptoms, and performance on a memory test (Randt Memory Test). Both ratings of quality of life and memory performance were improved in the ginseng/vitamin group. Similarly, a study by Wiklund *et al* (1994) demonstrated a more pronounced improvement from baseline in ratings of well being (Psychological General Well Being Index) for the 205 healthy participants in a ginseng/vitamin group as opposed to the 185 participants taking a placebo for 12 weeks. However, an examination of the efficacy of a ginseng containing complex (Gericomplex) in the treatment and rehabilitation of geriatric patients found no positive effect on any of the objective or subjective measures that were utilised (Thommessen and Laake, 1996). It should be noted that this trial suffered from serious methodological shortcomings (choice of population, duration of the trial, choice of endpoints) and its results cannot therefore be interpreted conclusively.

A further double-blind, placebo-controlled trial utilising a combination product (Ussher *et al,* 1995) also found no significant self-reported 'quality of life' improvements, in comparison to placebo, in 95 middle managers taking a ginseng/vitamins combination for 2 months. In common with the preceding studies this study utilised ginseng in combination with other compounds, making the attribution of experimental effects difficult. However a number of studies have administered ginseng alone, or attempted to isolate the effects of ginseng administered with other compounds. These include a double blind study by Wiklund *et al* (1999), using the same primary endpoint as their previous (1994) study, which demonstrated significant improvements in comparison to placebo on several sub-scales of the Psychological

General Well Being Index , but not on the whole index, following 16 weeks administration of ginseng (or placebo) to 394 symptomatic postmenopausal women. This finding is offered some qualified support by the results of an inadequately controlled trial by Tode *et al* (1999), which showed that 12 postmenopausal women with climacteric syndrome showed improvements both in an imbalance of hormones, and on measures of mood following 30 days administration of 6 g of ginseng. However, whilst suggestive, this result is difficult to interpret as the control group utilised was a cohort of 9 postmenopausal women without climacteric syndrome, who were offered no treatment.

The effects of ginseng has also been studied in non-insulin-dependent diabetes mellitus (NIDDM) patients. Sotaniemi *et al* (1995) in an 8 week double blind, placebo controlled study, investigated the effects of two doses (100 mg, 200 mg/day) of ginseng on 36 NIDDM patients. They demonstrated improvements in self ratings of mood, vigour and well being, as well as improved performance on a psychophysical test (timed diagram drawing), and reductions in fasted blood glucose levels, in comparison to placebo. There was, however no improvement on a working memory test (digit span). In keeping with the physiological response to *Panax ginseng* reported in the previous study, Vusksan *et al* (2000a; 2000b; 2001) demonstrated reductions in post-prandial blood glucose levels in both normal participants and non-insulin-dependent diabetic patients following administration of *Panax quinquefolium.*

Several studies have addressed the effects of ginseng in healthy populations. These include a study by Marasco *et al* (1996), who attempted to isolate the effect of ginseng on the well being of subjectively stressed and fatigued participants (n=625), in a double-blind study of the effects of either multi-vitamin capsules, or multivitamin/ginseng capsules taken for 12 weeks,. Both treatments induced a significant increase in a quality of life index in comparison to placebo, but the increase was significantly higher for the ginseng/vitamins group. A further study (Forgo and Kirchdorfer, 1981) compared ginseng (100 mg G115 twice daily) to placebo, and demonstrated, as a consequence of ginseng ingestion, a significant increase in pulmonary function and subjective assessments of well being for both younger (30-39yrs) female, and older (40-60 yr)

male and female groups. There was also an improvement in reaction times for the older male and female groups.

One study (Hallstrom *et al* 1982) investigated the role of ginseng in work related fatigue, with a double blind crossover study involving 12 night nurses. The two conditions involved administration of 1200 mg ginseng or placebo during night work, with a further comparison made with day time work. Measures included self-rating scales (mood, lethargy, sleep quality), psycho-physiological performance tests (tapping and cancellation tests), and haematological and biochemical tests. Night duty in itself impaired performance on all of the mood and most of the somatic measures. There were no significant changes in the ginseng group on any of the measures, except for improved performance on the tapping test, and deterioration in sleep quality and duration.

*Human ergogenic benefits*

The ergogenic effects of ginseng have been investigated in a number of studies. Methodological difficulties make interpretation of the results of several of the studies difficult. For instance, a study by Knapik *et al* (1983) demonstrated no effects but had a very small sample size (5 ginseng, 6 placebo); Pieralisi *et al* (1991) demonstrated substantial ergogenic effects, but for ginseng combined with dimethylaminoethanol bitartrate, vitamins, minerals and trace elements; and in two separate studies Forgo and Kirchdorfer (1981; 1982) demonstrated significant aerobic capacity, lactate level, and heart rate effects, but failed to include either placebo or control conditions.

Forgo (1983) did, however, extend the latter investigations with a double-blind, placebo-controlled investigation into the effects of 9 weeks of administration of either ginseng (G115), or ginseng plus tocopherol, or placebo, on the physical performance and hormone levels of athletes. He reported significant increases in oxygen uptake, and significant decreases in both exercise blood lactates and heart rate, but no change in hormone levels, for both of the active treatments in comparison to placebo. This was followed by a further double blind study (Forgo

and Schimert, 1985) investigating the duration of the effects of 9 weeks administration of ginseng (G115 - 100 mg twice daily) during exercise. Results reported include significant increases in oxygen uptake, forced expiratory volume and vital capacity, and significant decreases in heart rate and visual reaction times. Some of these differences persisted at testing three weeks after cessation of treatment.

Several recent studies do not, however, offer any support to this role of ginseng as an ergogenic aid. Morris *et al* (1996) in a placebo-controlled, cross-over study, found that 1 weeks administration of two different doses of ginseng had no more effect on any of the physiological indices under investigation (oxygen, free fatty acids, lactate, glucose) than placebo. Allen *et al* (1998) reported, in a randomised, double-blind, placebo-controlled study involving 28 healthy young adults, that the administration of 200 mg ginseng extract for 21 days did not significantly affect heart rate or perceived exertion at 150 and 200 watts ergometric exercise; and that it did not affect $VO_2$, exercise time, workload, plasma lactate or haematocrit at peak levels of exercise. Similarly, Engels and Wirth (1997), again in a randomised, double-blind, placebo-controlled trial involving 36 healthy men, failed to demonstrate any effect of 8 weeks administration of ginseng on $O_2$ consumption, respiratory exchange ratio, minute ventilation, blood lactic acid levels, heart rate or perceived effort. Bahrke and Morgan (1994, 2000) suggest that the equivocal nature of the evidence pertaining to the putative ergogenic benefits of ginseng can be attributed in the most part to methodological problems, including ineffectively controlled experimental paradigms and small sample sizes.

### 1.3.3.2 Cognitive effects

***Animal studies***

A number of studies suggest that ginseng can be effective in the attenuation of learning deficits due to brain damage and ageing in rodents. Examples include a demonstration, following 5 minutes of forebrain ischaemia in gerbils, of both neuro-protective properties (i.e. rescue of hippocampal CA1 pyramidal neurons) and amelioration of learning deficits (passive avoidance

step down) as a consequence of 7 days administration (prior to ischemia) of red ginseng powder, crude ginseng saponins, and ginsenoside $Rb_1$. A lesser effect was observed for crude ginseng non-saponins, and no effect was observed for ginsenosides $Rg_1$ and Ro (Wen *et al,* 1996). In a similar vein, Zhong *et al* (2000) demonstrated the same pattern of spatial learning deficits in aged rats and young rats with selective hippocampal lesions (in comparison to young rats and young sham rats respectively). In both cases learning decrements were attenuated by administration of Red ginseng powder. Similarly, a dose dependent attenuation of learning deficits in brain lesioned (medial prefrontal cortex) rats, and significant strategic learning improvements in sham control rats, both in comparison to saline controls, have been shown as a consequence of 30 days post-operative administration of 40 and 80 mg/kg crude ginseng extract (Zhao and McDaniel, 1998). Age related deterioration in performance on a radial maze task has also been attenuated by administration of ginseng extract. No such effect was evident, however, on an operant discrimination task (Nitta *et al,* 1995).

Ginseng related improvements in the learning and memory of normal and young rats tend to be both dose-dependent and sensitive to the nature of the task. As an example, Petkov and Mosharrof (1987) administered mice with 3, 10, 30, 100 and 300 mg/kg G115 and found an inverted-U dose/response relationship on some tasks, with 10 mg the most effective in facilitating 'shuttle box' active avoidance learning, whilst 30 mg significantly improved retention of 'step down' passive avoidance. However, only the 10 mg dose improved performance on staircase maze training with positive (alimentary) reinforcement, whilst 100 mg increased locomotor activity. Similarly Petkov *et al* (1993) demonstrated variations in learning as a function of method of assessment, age of rats and dosage (17, 50 or 150 mg/kg G115 for 7 days). So, for instance, young rats showed the greatest improvement in retention of 'shuttle box' passive avoidance with the lowest and highest doses (17 and 150 mg/kg), but only showed significant improvement on 'step down' passive avoidance with the middle dose (50 mg/kg), whilst neither 'step through' passive avoidance nor water maze learning were significantly affected by any dose of G115. Similarly, Petkov *et al* (1992) demonstrated favourable effects of

both leaf and root ginseng extracts on performance of active avoidance (shuttle-box), passive avoidance (step-down, step-through), and water-maze learning tasks for rats of varying ages. However, it was noted that these effects varied greatly with the dose and administration schedules, with the strain of rat, with the rat's ability to perform adequately in any particular learning task, and with the behavioural method employed.

Over time rodent research is tending more towards an examination of individual ginsenosides and fractions of whole ginseng. Evidence (Wen *et al,* 1996) suggests a role in animals' learning and memory for individual ginsenosides and specific fractions, e.g. a polysaccharide fraction (Lyubimov *et al,* 1997), and the water soluble fraction of an ethanol extract of ginseng (Nitta *et al,* 1995).

It is also particularly noteworthy that different fractions or doses of ginseng extract have been shown to impair learning. For instance, Saito *et al* (1977) found that extracts of ginseng inhibited conditioned avoidance response and discrimination behaviour on pole climbing and shuttle box tests. Similarly, Petkov and Mosharrof (1987) found that high doses of ginseng impaired rather than improved conditioned reflex activity, and Takagi *et al* (1972a; 1972b) demonstrated decreased exploratory activity, and a specific blocking action of conditioned responses, following administration of a crude saponin fraction.

Interestingly, Smriga *et al* (1995), in an investigation into the individual ingredients in the putatively nootropic Chinese prescription DX-9386, found that a single oral administration of ginseng (500 mg/kg) significantly increased hippocampal long term potentiation (LTP) in anaesthetised rats. This finding was broadly in line with similar demonstrations *in-vivo* of modulation of LTP in the hippocampal formation by ginsenoside $Rb_1$ (Abe *et al,* 1994), and unpublished observations of *in-vitro* enhancement of LTP in the CA3 subfield of the hippocampus by an aqueous extract of ginseng (Zhong *et al,* 2000).

***Human studies***

Whilst there is a good body of work attesting to the cognition enhancing effects of ginseng with regard to animals, the evidence is scarce with regard to humans.

Several of the studies outlined above assessing 'adaptogenic' effects included a cognitive element. For instance, Neri *et al* (1995) found improved mnemonic performance (Randt Memory Test) in their cohort suffering from age related memory impairment, whereas Thommessen and Laake (1996) found no improvement in geriatric patients on performance of the Mini-Mental State Examination, the Kendrick Object Learning test or the trail making test. Non-insulin-dependent diabetic patients exhibited an improvement in psychophysical performance (timed diagram completion) but no memory improvement (digit span test) (Sotaniemi *et al* 1995), whilst Forgo and Kirchdorfer (1981) reported an improvement in reaction times that was restricted to older (40-60) male and female participants.

Only two investigations have focussed directly on the effect of chronic administration of ginseng on cognition. Both employed a double-blind, placebo-controlled design. The first study was that by D'Angelo *et al* (1986) involving 32 healthy young (20-24) volunteers who were given either 100 mg of G115® or placebo twice a day. Testing took place prior to and following 12 weeks of treatment. Tests included those assessing motor performance (finger tapping), auditory and visual simple reaction times, choice reaction times, attention (digit cancellation and digit symbol substitution), mental arithmetic performance, and logical deduction performance. Within-groups analysis showed that performance in the ginseng, but not the placebo, group was significantly improved above baseline on choice reaction time, logical deduction and cancellation tests. However, between groups analysis revealed that performance was only significantly improved for the ginseng group in comparison to placebo on the mental arithmetic test, which involved calculation of whether the sum of four two digit numbers was odd or even.

The second study was by Sorensen and Sonne (1996) and involved 112 healthy participants over 40 yrs (40-70) who received either 400 mg of standardised ginseng extract or placebo

daily for 8 to 9 weeks. Tests included the finger tapping test, both auditory and visual simple reaction time tests, a 5 minute letter and symbol cancellation test, a verbal fluency test (as many animals as possible in 1 minute), a Logical Memory and Reproduction Test (reproducing units of linguistically meaningful information), the Rey-Oestrich Complex Figure Test, and a computerised Wisconsin Card Sort Test. Results showed statistically significant performance improvements for the ginseng group, in comparison to placebo, only on the fastest trials of the auditory simple reaction time tests, and on the Wisconsin Card Sort Test.

It is particularly interesting to note that by convention, but with no underlying scientific rationale, studies into the effects of ginseng on humans have invariably involved chronic regimens of several weeks or months.

### 1.3.4 Conclusion

It seems that ginseng exerts a number of physiological effects. In itself, the wealth of research indicating both microscopic and macroscopic physiological effects would suggest that ginseng may be psychoactive. However, its behavioural or psychological effects remain to be elucidated.

Whilst there seems to be some evidence of both 'adaptogenic' and mnemonic effects in animal studies, results are at times both variable and equivocal. In humans there is some evidence, largely from poorly controlled trials, that suggests that ginseng is an ergogenic aid. On the other hand an equal amount of evidence suggests that it is not. Similarly, positive results with regard to 'well being', reaction times and mnemonic improvement, tend to be isolated and variable and accompanied in individual studies by negative results on other measures.

There are several possible reasons for this lack of clarity in the literature.

The first is, necessarily, the qualitative and quantitative nature of the treatments administered. Animal research typically utilises a wide range of doses, with animals being administered dosages per kilogram (from 3 to 300 mg/kg/day) far in excess of anything given to humans (200 mg to 2 grams/day). This is particularly pertinent in light of the dose dependent effects

demonstrated in a number of studies utilising adequately standardised ginseng extracts (e.g. Petkov and Mosharrof, 1987; Petkov *et al,* 1993; Petkov *et al,* 1992). Similarly, a huge range of ginseng extracts, combinations, fractions and single and multiple ginsenosides, have been utilised. This could feasibly generate any number of single, multiple, contradictory, and synergistic effects. Bahrke and Morgan (1994) note that in animal research, *'hypertensive and hypotensive effects, hystamine and antihystamine like actions, and both stimulant and depressant activity on the central nervous system have been reported'*(p232). This point is particularly pertinent in light of recent evidence further delineating the disparate effects both of the major groups of ginsenosides (e.g. Kudo *et al,* 1997; Tachikawa *et al,* 1999; Yamaguchi *et al,* 1995, 1996), and of extracts differing in their concentration ratios of panaxadiols and panaxatriols (Jin *et al,* 1999). Whilst human research has almost exclusively been undertaken with whole extracts of *ginseng,* this final point is still relevant in light of both the wide variability of ginsenoside content of commercially available *ginseng* products (Cui, 1995), and the differing ginsenoside contents of extracts as a function not only of the species of ginseng, but also with the age of the plant, manner of cultivation, and the drying and curing process (Phillipson and Anderson, 1984; Smith *et al,* 1996).

The second confounding element in the literature is the methodological limitations of much of the research undertaken on humans. Research design is a pertinent example. Bahrke and Morgan (1994) note that human ergogenic research is typified by an absence of appropriate variables and control groups and/or control trials. They note that as a consequence *'most blind/placebo controlled trials have yielded negative results..... whereas positive results have been observed where blind/placebo controls have not been employed'*(p243). In a recent update on their previous review Bahrke and Morgan (2000) note that the literature published in the intervening period has failed to clarify the equivocal nature of the evidence. On a similar note Vogler *et al* (2000), in a review of randomised controlled ginseng trials, conclude that the evidence from the few adequately controlled trials that met their inclusion criteria (16 out of a total of 57 retrieved), was not compelling for the efficacy of ginseng with regard to any

indication for which it might be taken. The most pertinent conclusion that might be drawn from this is that, given the huge number of research papers published on ginseng (a search of Medline using the single search term 'ginseng' returns over 1400 journal articles), the vast majority of which concern themselves with *in vitro, in vivo* and animal research, it would seem to be timely to direct more quality research to the actual effects of *ginseng* in humans.

The most pertinent conclusion that can be drawn from the literature pertaining to ginseng and cognition is that whilst ginseng undoubtedly affects cognition, the question of whether these effects are, on balance, beneficial, remains to be elucidated through appropriately controlled investigations of the effects of standardised extracts, utilising adequately sophisticated and sensitive psychometric instruments.

## 1.4 Ginkgo biloba/Panax ginseng combination

### 1.4.1 General information

Whilst the examination of the cognitive effects of combined herbal products is generally outside the scope of the current thesis, one such combination bears investigation, being made up, as it is, from standardised extracts of both *Ginkgo biloba* and *Panax ginseng*. Whilst a number of manufacturers offer products containing the two herbs, the product that has attracted a certain amount of research interest is a direct combination of two 'gold standard' extracts; *Ginkgo biloba* (GK501) and *Panax ginseng* (G115). These are combined in a ratio of 60:100 in 160 mg capsules marketed under the trade name Ginkoba® ME (in some countries Gincosan®). No direct research has been directed at the pharmacokinetics, or physiological *in vitro* effects of the combination *per se*. However, barring the possibility of a synergistic interaction, it seems expedient to assume that the foregoing reviewed research on the component extracts can be taken as an indication of the possible properties of the combination.

### 1.4.2 Mechanisms of action

***Effects on blood circulation***

In a similar experiment to a previous investigation of the haemorrheological and circulatory properties of *Ginkgo biloba* (Jung *et al,* 1990), Kiesewetter *et al* (1992) examined the effects of two single doses (160 mg and 320 mg) of the combination in 10 healthy young volunteers. Their findings included a reduction in platelet aggregation and systolic blood pressure following both doses, and further reductions in diastolic blood pressure, heart rate, and an increase in erythrocyte velocity in nail fold capillaries for the higher dose.

### 1.4.3 Cognitive effects

*Animal studies*

Petkov *et al* (1993) demonstrated dose-dependent improvements in retention of learned behaviour on a number of tasks for both young (3 months) and old (26 months) rats as a result of ingestion of extracts of ginseng, ginkgo and the product combining the two. The authors suggested that the combination may be a 'particularly promising drug in geriatric practice'.

*Human studies*

The effects of a *Ginkgo biloba/Panax ginseng* combination were assessed in a double-blind study utilising 64 participants who satisfied the criteria for neurasthenia (cerebral insufficiency), an age related condition with a possible cerebro-vascular aetiology, typified by fatigue and tiredness (Wesnes *et al,* 1997). Results from the CDR computerised test battery showed significant dose-dependent improvements on a 'Quality of Memory' index, one of a number of primary outcomes derived by factor analysis of the individual outcome measures from the whole test battery. Improvements were seen at 1 hour past the morning dosing on days 1, 30 and 90, but with a reversal of this effect following the afternoon dosing for the highest dose. Participants in the 160 mg/day group also exhibited augmented speed of responses following the morning dose throughout the study.

In order to extend this line of research, and clarify the possibility of a bi-phasic effect, a further, larger, double-blind study was undertaken (Wesnes *et al,* 2000). This study utilised a cohort of 256 healthy middle-aged participants randomly allocated to 3 conditions: 160 mg Gincosan twice daily; 320 mg Gincosan once daily, and placebo. Testing took place four times daily (1 hour pre-dose and 1, 3, and 6 hours after the first dose) at pre-commencement of treatment and at 4, 8, 12 and 14 weeks post-commencement of treatment.

Once again the primary outcomes were four cognitive factors derived from the complete CDR battery, and once again a significant improvement was demonstrated on the 'Quality of

Memory' factor, which comprises scores from 6 separate memory tasks. These improvements were evident at 1 hour and 6 hours (the latter of which corresponds to the testing session which evinced decrements in the first study) following the daily/first dose of Gincosan. No improvement was evident at 3 hours post-dose or at the pre-dose testing session. This lack of an improvement prior to the days treatment would seem to indicate that the enhancement may well have been as a result of the acute effects of the days treatments, rather than an accumulative effect of the ginkgo/ginseng combination over time. This latter possibility is strengthened by observation of a significant improvement on the 'Quality of Memory' factor 1 hour after the first dose on the first day of the original study (Wesnes *et al,* 1997).

What is particularly interesting about these two studies is the utilisation of an integrated computerised test battery, a tool which has never been used in ginseng research, and which has rarely been used in *Ginkgo biloba* research (the obvious exception here are studies by Rai *et al* (1991) and Wesnes *et al* (1987) that also utilised the CDR battery). Of particular interest is the use of factor analysis derived cognitive outcomes which combine a number of individual task outcomes, and which may well be of utility in detecting what are likely to be subtle differences associated with herbal remedies.

## 1.5 European Herbs

### 1.5.1 Background

As can be seen from the foregoing, both *Ginkgo biloba* and ginseng have generated a substantial body of research. It could be suggested that the specific overlying impetus that has shaped this body of work is slightly different for each. In the case of *Ginkgo biloba* interest was initially sparked, and then sustained primarily in European countries, by the delineation in the 1960's of a high quality, standardised product (Egb 761, Dr William Schwabe) that facilitated meaningful investigation. A reasonably well-balanced literature has since developed, driven forward by overwhelmingly positive results. The impetus for ginseng, on the other hand, would seem to be a largely Asian curiosity as to the physiological mechanisms underlying the known efficacy of a tried and tested traditional medical treatment. A huge extant literature exists, a sizeable majority originating from areas and communities that have long histories of ginseng usage, the majority of which concerns the microscopic and physiological *in vitro* and *in vivo* concomitants of its usage. Research, often methodologically flawed, into the effects in humans, has been largely undertaken in the West, with, as has been seen, only limited success. The question that this raises is, in a society that has attached 'added value' to synthesised rather than natural medicinal products for many decades, and therefore in the absence of an unbroken popular tradition of herbal usage, what potentially efficacious traditional European herbs have been overlooked.

This question has been addressed by Perry and co-workers (e.g. Mantle *et al,* 2000b; Perry *et al,* 1996; Perry *et al,* 1998; Wake *et al,* 2000). The over-riding rationale underlying their approach is that whilst traditional plants (with the exception of *Ginkgo biloba)* have largely been overlooked by western medicine in the treatment of dementia, there are a number of plant species reputed, over the centuries or indeed millennia, to have cognition and memory enhancing effects. These plants may well be efficacious in treating the specific biological mechanisms underlying dementia. In the case of Alzheimer's disease these mechanisms may include: disrupted cholinergic transmission; neuronal damage caused by oxidative stress and inflammatory reactions; and beta-amyloid formation or toxicity (Perry *et al* 1999). With this in

mind a sensible approach is to investigate the properties of traditionally utilised European plants with regard to potential utility in these domains. To this end a number of *in vitro* investigations of European plants' properties have examined, for example, anti-oxidant properties (Mantle *et al* 2000a), and both acetylcholinesterase inhibition (Perry *et al* 1996), and cholinergic receptor interactions in human brain tissue (Perry *et al* 1996; Wake *et al* 2000).

The results of such investigations suggest that three specific members of the Labiatae family: *Salvia (officinalis* and *lavandulaefolia)* (sage)*; Melissa officinalis* (lemon balm)*;* and *Rosmarinus officinalis* (rosemary) have a long tradition in the treatment of disorders of the central nervous system or memory enhancement. In the case of sage and melissa they also possess *in vitro* properties that suggest not only a possible role in the treatment of the cognitive decline associated with dementia, but also, by definition, in the general cognitive enhancement of healthy populations. On the basis of the strength of evidence pertaining to these herbs an investigation of the effects on cognition of two of these plants, *Salvia lavandulaefolia* (sage) and *Melissa officinalis* (lemon-balm), forms the second part of this thesis. The evidence suggesting a possible role in cognition enhancement is reviewed below.

### 1.5.2 Sage

#### *1.5.2.1 General information*

*Salvia*, with over 700 species, is the largest genus in the Labiatae family. Whilst the genus was recognised and named by both Egyptian and Greek civilizations it owes its name to the Romans (from the Latin salvare - 'to save') (Rivera *et al,* 1994).

*Historical perspective*

Perry *et al* (1999) note that the genus has been used independently across the millennia in a number of distinct cultures. So, for example, the Greeks considered garden sage, ('elelisphakon' – *S. officinalis)* to be '*good for helping diminution of senses and loss of memory*' (Ryman, 1991), Ayurvedic medicine, one of the longest surviving systems of herbal medicine, prescribes

its use to '*clear emotional obstructions from the mind and for promoting calmness and clarity*' (MacIntyre, 1996), and a number of *Salvia* species have been used in traditional Chinese medicine, with the genus, described as a 'superior' herb, appearing in the *Shen Nung pen tsao* (25BC). Species include *S. plebeia, S. chinensis* and *S. miltiorrhiza* (Dan shen), the last of which is reputed to invigorate blood circulation, cool and nourish the blood and calm mental irritability (Hsu *et al,* 1986). Interestingly, the use of indigenous varieties of sage was supplemented with *S. officinalis* following contact with Europeans in both American Indian (Moerman, 1986) and Chinese medicine, with, in the latter case, an exchange rate in the Orient of 3:1 finest tea to European sage tea (Perry *et al* 1998).

The Salerno medical school, the first in medieval Europe, considered locally grown *Salvia fructiosa* to be a 'Herba sacra' (sacred herb), which was described as a cure with a calming effect, and which featured in the proverb in the 'Tabuli Salerni', ' *Cur moritur, qui salvia crescit in horto*' (Why should he die who has sage in his garden?) (Valnet, 1990). It seems likely that at the same time *S. officinalis* was in common usage throughout Europe, and it is particularly germane to note that it has featured in British herbal apothecaries from the 16$^{th}$ century onwards (Crellin and Philpott, 1990). Perry *et al* (1999) note that during this epoch *S. officinalis* was recognised as an enhancer of memory, and they provide quotations from some of the foremost Herbals of the day. So, for instance; Gerard, in 1597, suggests that '*It is singularly good for the head and brain and quickeneth the nerves and memory*'; Culpepper's 'Complete Herbal' notes, in 1652, that '*It also heals the memory, warming and quickening the senses*'; and John Hill's 'The Family Herbal' tells us, in 1756, that, '*Sage will retard the rapid progress of decay that treads upon our heels so fast in latter years of life, will preserve faculty and memory more valuable to the rational mind than life itself*'.

It is worth noting that throughout its history the naming of the species of the genus has often been confused, and even today *S. officinalis* is conventionally thought of as the commercial herb, whereas, in reality, the majority (50-95%) of sage imports to, for instance, the USA are in fact *S. libanotica* (East meditteranean sage) (Gali-Muhtasib *et al,* 2000).

*Contemporary usage*

Specific contemporary indications for *S. officinalis* include: use as a gargle or mouthwash as a treatment for inflammation of the mouth, tongue or throat; alleviation of flatulent dyspepsia, and loss of appetite; reduction of blood sugar; treatment for cases of respiratory allergy, headache, anxiety and nervousness in the elderly; poor memory, mental confusion, depression, vertigo and as a treatment for the symptoms of the menopause (Bartram, 1998; British Herbal Pharmacopoea, 1983).

*Possible active components*

Essential oil of *Salvia officinalis* contains alpha- and beta-thujone as major components (around 50%). It also contains cineole, borneol and camphor, a number of flavonoids (Leung and Foster, 1996), and a number of polyphenolic compounds, most notably (in order of concentration as assessed by densitometry), rosmarinic acid, methyl carnosate, caffeic acid, luteolin 7-0-glucoside, and luteolin (Hohmann *et al* 1999).

Whilst *Salvia lavandulaefolia* (Spanish Sage) contains similar components, it lacks the thujone content. Thujone is toxic, and ingestion of large doses causes convulsions (Leung and Foster, 1996). Whilst *S. officinalis* oil does not appear to be as hazardous as its thujone content might warrant (Price, 1998), it has been suggested that *S. lavandulaefolia* may provide an equally efficacious, but more theoretically suitable, treatment (Mantle *et al*, 2000).

In terms of the whole herb, both sage species contain about 1.0 – 2.8 % volatile oil (Leung and Foster, 1996).

### *1.5.5.2 Mechanisms of action*

*Acetylcholinesterase inhibition*

The only current treatments for Alzheimer's disease approved in the UK are drugs that inhibit either the cholinesterase (ChE) group of enzymes, or specifically acetylcholinesterase (AChE), in both cases leading to an augmentation in the availability of the neurotransmitter acetylcholine. They are attributed with a '*significant, although modest, effect on the cognitive status of patients with AD*' (Grutzlender and Morris, 2001), and include the plant (snowdrop and daffodil bulb) derived drug galantamine, which has proved effective in clinical trials (e.g. Wilcock *et al,* 2000) and was recently licensed.

Several terpenoids that have been shown to inhibit AChE occur in the *Salvia* genus (Miyazawa *et al,* 1997; 1998), and concentration dependent inhibition of AChE in human brain homogenates was demonstrated *in vitro* as a consequence of the application of the essential oil of both *Salvia officinalis* and *S. lavandulaefolia* (Perry *et al,* 1996). Whilst anti-cholinesterase activity was also demonstrated following application of alcoholic extracts of both fresh and dried herb, none of the individual constituents tested (borneol, caffeic acid, camphor, cineol, and thujone) inhibited the enzyme, suggesting another unidentified constituent was responsible. In a similar vein, Perry *et al* (2000) demonstrated dose dependent inhibition of erythrocyte AChE by *Salvia lavandulaefolia* essential oil, but found that no single constituent was particularly potent, suggesting a synergistic relationship. This *in vitro* anti-cholinesterase activity of the essential oil of *S. lavandulaefolia* has also been confirmed *ex vivo,* with the demonstration of a similar effect as physostigmine on the contractile response of the isolated guinea pig ileum (Perry *et al,* 2001), and *in vivo* with inhibition of AChE in selected brain areas following oral administration of *S. lavandulaefolia* to aged rats (Perry *et al,* 2002). This last report is particularly important as it demonstrates that the active components of the essential oil are capable of surviving digestion, and traversing the blood brain barrier for delivery to therapeutically relevant sites.

*Anti-oxidant and anti-inflammatory properties*

Oxidative stress plays a role in the general processes of ageing and tissue damage, and is implicated in the pathogenesis of Alzheimer's disease (AD) (Markesbery, 1997). Similarly, a role in AD aetiology has been postulated for inflammatory mechanisms (Aisen, 1996).

Lamaison *et al* (1993) demonstrated that many species of Labiatae, including *S. officinalis* exhibited free radical (1,1-diphenyl-2-picrylhydrazyl) scavenging properties.

Both *S. officinalis* and *S, lavandulaefolia* have been shown to have a number of anti-oxidant components (e.g. Djarmati *et al,* 1992; Dorman *et al,* 1995). In an investigation of the *in vitro* anti-oxidant properties of a number of British medicinal plants Mantle *et al* (2000a) demonstrated that *Salvia officinalis* leaf had 'appreciable' levels of anti-oxidant activity (0.32 mmolTE/gm dry weight), in comparison to recognised antioxidants such as *Ginkgo biloba* and *Panax ginseng* (0.62 and 0.61 mmolTE/gm dry weight respectively). This result is in line with research by Hohmann *et al* (1999) who demonstrated that, on the basis both of antioxidant properties and proportional contents of its several polyphenolic components, that the antioxidant properties of a whole extract of *S, officinalis* is probably largely attributable to its Rosmarinic acid content. Anti-oxidant properties have also been confirmed in *S. Lavandulaefolia* (Perry *et al*, 2001).

Support for a traditional role for *Salvia* species as anti-inflamnmatories (Bartram 1995), was offered by the demonstration of anti-inflammatory actions by a *S. Lavandulaefolia* extract and several constituents (Perry *et al*, 2001).

*Oestrogenic properties*

Several studies indicate that oestrogen replacement therapy is associated with both lowered risk of Alzheimer's disease, and amelioration of symptoms in Alzheimer's disease (Henderson 1997), although the evidence is not equivocal in this respect (e.g. Mulnard *et al,* 2000).

Oestrogen receptor stimulation can produce a number of different pharmacological effects (Jordan, 1998). In line with this, oestrogen has been shown to have a number of CNS effects, including increased cerebral blood flow, anti-inflammatory actions, enhancement of activity at neuronal synapses, and neuroprotective and neurotrophic effects on brain tissue (Shepherd, 2001). Contemporary uses of Sage include its use as a component of preparations for the treatment of gynaecological problems (Bartram, 1995), and, in the UK, as an over the counter treatment for the relief of symptomatic disturbances associated with the menopause (e.g. Holland and Barrett dried Sage leaf capsules). This role receives cautious support from an open trial showing the amelioration of menopausal symptoms in 30 women by a product containing extracts of *S. officinalis* and *Medicago sativa* (alfalfa) (De Leo *et al,* 1998). In line with suggestions that members of the *Salvia* genus may have oestrogenic properties (Perry *et al,* 1998), Perry *et al* (2001) demonstrated oestrogenic activity in the essential oil of *S. lavandulaefolia* , and the monoterpenoid component *geraniol.*

### 1.5.3 Melissa Officinalis

#### *1.5.3.1 General information*

Another member of the Labiatae family, *Melissa Officinalis* (lemon balm), is a cultivated perennial lemon scented herb. Approximately 50 tons of balm leaves are sold each year for medicinal purposes in Germany alone, much of which is cultivated in Eastern European countries and Spain.

*Historical perspective*

Records concerning the use of *M. officinalis* date back over 2000 years with entries in the 'Historia Plantarum' (approximately 300 BC) and the 'Materia Medica' (approximately 50-80

BC). From its Moorish introduction into Spain in the seventh century its cultivation and use spread throughout Europe by the Middle Ages (Koch-Heitzmann and. Schultze, 1988). Medicinal use throughout this early epoch include a recommendation by Paracelsus (1493-1541) that balm would completely revivify a man, and indication for "*all complaints supposed to proceed from a disordered state of the nervous system*" (Grieve, 1931). Several herbal Apothecaries of the time also attributed balm tea not only with general beneficial effects upon the brain, but also with specific mnemonic improvements (Coghan, 1584; Evelyn, 1699).

*Contemporary usage*

Contemporary reports stress the sedative, spasmolytic, and antibacterial effects of *Melissa officinalis*, with indications encompassing nervous disorders, gastro-intestinal disorders and sleep disturbance (Bisset and Wichtl, 1994; Kommission E Monograph, 1984). In keeping with its long history of safe usage no side effects have so far been reported (Wong *et al,* 1998).

*Melissa officinalis* is predominantly sold in combination with other herbs. As an illustration, 49 products containing lemon balm are included in the German pharmaceutical industry's 'Rote Liste' (2001) drug catalogue.

*Possible active components*

A number of possible active components of the dried leaf and essential oil of the herb have been identified. Constituents that may have pharmacological effects include a number of monoterpenoid aldehydes (including Citronellal, Neral and Geranial), (Carnat *et al*, 1998), flavonoids and polyphenolic compounds (most notably rosmarinic acid) (Carnat *et al*, 1998; Hohmann *et al,* 1999) and monoterpene glycosides (Mulkens *et al*, 1985).

Variations in the percentage content of individual components in balm oil can be attributable to a number of causes. For instance, varying origins and harvest times, the number of years of

cultivation, developmental stage of the specific harvested leaf, the use of fresh or dried material, the nature of the drying and oil extraction process, and the duration of the storage of the plant product (Tittel *et al,* 1982).

### *1.5.3.2 Mechanisms of action*

*Acetylcholinesterase inhibition*

Two commercial *M. officinalis* oils were found to have substantial acetylcholinesterase inhibiting properties in human brain homogenates (Perry *et al* 1996). However, this finding has to be treated with caution as lime oil, which is often an added ingredient in commercial balm preparations, exhibited similar properties. Fresh leaf, but not dried leaf, also exhibited modest anti-cholinesterase activity with a % inhibition (26.4 % inhibition at 2 mg leaf/ml) of something over half that exhibited by *Salvia officinalis* fresh leaf (47% inhibition at 2 mg leaf/ml).

*Cholinergic receptor binding properties*

*Melissa officinalis* exhibited central nervous system acetylcholine receptor activity, with demonstrations of both nicotinic (Perry *et al,* 1996; Wake *et al,* 2000) and muscarinic (Wake *et al,* 2000) binding properties in homogenates of human brain tissue. In the case of the latter study, six separate accessions of melissa leaf elicited markedly different proportions of binding to the two acetylcholine receptor subtypes in human occipital cortex tissue, with $IC_{50}$ concentrations ranging from 0.08 mg to 3.8 mg/ml for the displacement of [$^3$H]-(N)-nicotine from nicotinic receptors, and from 0.5 to >5 mg/ml for the displacement of [$^3$H]-(N)-scopolamine from muscarinic receptors.

*Anti-oxidant and anti-inflammatory properties*

It has been demonstrated that many species of Labiatae, including *M. officinalis* exhibit free radical (1,1-diphenyl-2-picrylhydrazyl) scavenging properties (Lamaison *et al,* 1993; Tagashira and Ohtake, 1998).

In an investigation of the *in vitro* anti-oxidant properties of a number of British medicinal plants Mantle *et al* (2000a) demonstrated that *Melissa officinalis* leaf had modest but 'appreciable' levels of anti-oxidant activity (0.18 mmolTE/gm dry weight), in comparison to recognised antioxidants such as *Ginkgo biloba* and *Panax ginseng* (0.62 and 0.61 mmolTE/gm dry weight respectively). This result is in line with research by Hohmann *et al* (1999) who demonstrated that, as with *S. officinalis,* on the basis both of inhibiton of enzyme dependent, and independent, lipid peroxidation and the proportional contents of its several phenolic components, the antioxidant properties of a whole extract of *M, officinalis* is probably mainly attributable to its Rosmarinic acid content.

#### *1.5.3.3 Animal studies*

In line with its contemporary role as a mild sedative, a number of studies involving rodents suggest specific 'calming' or sedative effects. Examples include a reduction in spontaneous movement demonstrated in mice as a consequence of both the whole volatile oil of melissa and the individual isolated terpenes, with prolongation of hexobarbital induced sleep in mice after even the lowest dose (1mg/kg) of oil administered orally (Wagner and Sprinkmeyer, 1973). Similarly, reductions in behavioural parameters in mice on both familiar and non-familiar environment tests were elicited by an hydro-alchoholic extract of *M officinalis,* but not by essential oil (Soulimani *et al,* 1991). An inverted U shaped dose response was evident with the greatest effect following 25 mg/kg (dose range 6-100 mg/kg). The plant extract also increased pentobarbital induced sleep parameters. This effect was also later observed to follow an inverted U dose response pattern with the soporific effect increasing up to 15 mg/kg then decreasing at doses between 200 and 400 mg/kg (Soulimani *et al,* 1993).

#### *1.5.3.4 Human Studies*

Whilst no studies have looked at the effects of melissa alone on humans, several have investigated the effects of a valerian/melissa combination on sleep quality, with, for example,

similar improvements demonstrated as those associated with 0.125 mg of Triazolam in poor sleepers, but with no rebound effects noted (Dressing *et al,* 1992). Similarly, significant improvements in quality of sleep were demonstrated for 66 healthy participants in a 360 mg valerian/240mg melissa condition, in comparison to a placebo group, over a 30 day dosing period (Cerny and Schmid, 1999).

### 1.5.4 European Herbs Conclusion

Considering the convergence of historical evidence of usage as a general treatment for the 'brain' or memory, which is evident across a number of independent cultures, and in light of research hinting at the possibility of relevant pharmacological actions, it seems entirely plausible that both *Salvia lavandulaefolia* and *Melissa officinalis* may provide novel treatments for Alzheimer's disease (Mantle *et al,* 2000b; Perry *et al,* 1998; Perry *et al,* 1996).

In both cases extracts have been shown to have properties that may ameliorate the cognitive disturbances associated with the basal forebrain cholinergic neuron loss implicated in the disease, and the consequent decline in acetylcholine and nicotinic receptor populations (Perry, 1986). In the case of *M. officinalis* the most likely putative mechanism would be direct binding to cholinergic receptors in the brain (Perry *et al,* 1996; Wake *et al,* 2000), whilst, with regard to *S. lavandulaefolia,* the potential relevant mechanism is an inhibition of acetylcholinesterase, with a consequent increase in synaptically available acetylcholine, a possibility that is suggested not only *in vitro,* but also both *ex vivo* and *in vivo* (Perry *et al* 1996; 2000; 2001). This last mechanism is the basis for the single action drugs that are currently the only available treatment, and of particular interest is the possibility that the additional properties of the plant extracts (and indeed the lack of side effects associated with either plant extract) may add another dimension to treatment. These 'added ingredients' include antioxidant properties in

both cases, particularly germane given the possibility not only that amyloid itself may be a source of free-radical damage, but also that oxidative damage is implicated in the pathogenesis of the disease (Hensley *et al,* 1995, Markesbery, 1997). Components of *S. lavandulaefolia* may also interact with oestrogen receptors, which opens the way for a number of potentially beneficial effects, including increased cerebral blood flow, anti-inflammatory actions, and neuroprotective and neurotrophic effects on brain tissue (Shepherd, 2001). *M. officinalis* also putatively possesses calming properties (Soulimani *et al,* 1991; 1993) that may serve to concurrently ameliorate the extreme agitation exhibited by Alzheimer's sufferers.

Whilst the fight against the cognitive disturbances implicit in Alzheimer's disease does not specifically concern the current thesis, this investigation into the effects of sage and melissa will nevertheless represent the first controlled, scientific examination of the cognitive effects of either plant extract in humans. Both exhibit properties that could lend them a role in a number of pathological conditions, and each of these demonstrated properties should also, theoretically, be capable in itself of modulating cognitive performance in healthy populations. With this in mind the current thesis's investigation of these two plant species may represent a first step towards clinical use of the extracts, and may provide preliminary information not only on cognitive effects, but also on effective doses, and tolerability in humans.

## 1.6 Other putative cognition enhancers

### 1.6.1 Background

Whilst it is not possible, for both practical and theoretical reasons, to investigate the effects of every herbal remedy that has been attributed with enhancement of some aspect of cognitive performance, the following comprises a necessarily brief synopsis of the evidence pertaining to some of the preparations that have not been included in the foregoing. Whilst the actual plant extracts investigated in the current thesis could be said to have self-selected themselves either on the weight of research/evidence, or alternatively because of a particularly compelling rationale for their inclusion, the following herbal preparations have been excluded for several specific reasons. A number of single ingredient extracts have been excluded on the basis either that they are not generally available on the European market (e.g. Brahmi, Peony root), or that they are under scientific scrutiny predominantly for other indications (e.g. St John's Wort). Multiple ingredient preparations, on the other hand, have been excluded simply on the basis that, given limited time and resources, experimental effects that cannot be attributed to single ingredients are to a certain extent theoretically uninformative. The obvious exception to this is the inclusion of the ginkgo/ginseng combination which achieves inclusion on the basis that it is widely commercially available, has already attracted some research interest, and is composed of two standardised ingredients both of which are themselves included. This raises the possibility of investigating the additive, or synergistic effects of the two.

The following also represent herbal preparations or remedies, rather than treatments derived from plants. To elucidate the difference it is interesting to note that many of the following exhibit cholinergic activity, and their literature includes the attenuation of scopolamine induced learning deficits. However, galanthamine, physostigmine, and huperzine, are all AChE inhibitors, are all derived from plants (Daffodil bulbs, Calabar beans and Huperzia moss respectively), but they also represent a medical treatment extracted from plants, thereby crossing the medical and legislative threshold (the Medicines Act 1968 allows exemptions from licensing for a herbal remedy which is made up solely of the herb, and is supplied without

recommendations as to its use) away from being herbal remedies. As such they fall outside of the scope of the current thesis.

### 1.6.2 European traditional Medicine

***St John's Wort*** *(Hypericum perforatum)*

St John's Wort has a well-documented antidepressant activity, which has been attributed to numerous possible actions, including reuptake inhibiton of serotonin, noradrenaline, dopamine, GABA and glutamate, and modulation of the function of a number of neurotransmitter receptors (Nathan, 2001). Whilst no studies have, as yet, assessed the cognitive effects concomitant with such widespread CNS activity in humans, a number have addressed the question in rodents. For instance, Kalifa (2001) demonstrated memory enhancement in mice following single doses of St John's Wort. Concurrent manipulation of individual neurotransmitter function suggested that this facilitatory effect was due to the involvement of adrenergic and serotonergic 5-HT1A receptors.

### 1.6.3 Chinese and Japanese Traditional Medicine

***Peony root*** *(Paeonia suffruticosa)*

It has been suggested that both the attenuation of scopolamine induced mnemonic deficits (Watanabe *et al,* 1991) and the traditional memory enhancing properties of Japanese traditional medicine *Shimotsu-to,* a combination of four plant extracts, is due to the inclusion of peony root, and its active principal Paeoniflorin (Watanabe, 1993). In line with this, the amelioration of both scopolamine (Abdel-Hafez *et al,* 1998; Ohta *et al,* 1993), and age related (Ohta *et al,* 1994) mnemonic deficits, and reversal of the suppressive effects of scopolamine and pirenzepine on long term potentiation in the hippocampus of rats (Tabata *et al,* 2000) have been demonstrated. Cholinesterase inhibiting properties have also been established for constituents of the plant extract (Park *et al,* 1996).

***Japanese angelica root** (Angelica sinensis)*

Another ingredient of *Shimotsu-to*, Angelica root has also been shown to reverse scopolamine (Hsieh *et al,* 2000; Ohta *et al,* 1993) and cycloheximide (Hsieh *et al,* 2000) induced amnesia in mice.

***Gastrodia elata***

An ancient Chinese medicine that is most commonly prescribed for neurasthenic complaints, *Gastrodia elata* has been shown to have beneficial effects on cerebral blood flow, and to protect against hypoxia (Huang, 1985). Oral administration to rodents for 1 week has been shown to attenuate scopolamine induced learning and memory deficit in rats on a passive step through task (Hsieh *et al,* 2000; Wu *et al,* 1996a). Constituents of the plant also attenuated memory consolidation and retrieval deficits following administration of cycloheximide and apomorphine, but not acquisition deficits following scopolamine. This pattern of improvements was unlike that engendered by piracetam (Hsieh *et al,* 1997), but was similar to that reported by Wu *et al* (1996b), who demonstrated that an active ingredient of *Gastrodia elata* attenuated deficits on an inhibitory avoidance task associated with a serotonin releaser (p-Chloroamphetamine) and a dopaminergic receptor agonist (apomorphine), but not a cholinergic receptor antagonist (scopolamine).

***S-113m***

S-113m is a novel Chinese herbal prescription consisting of *Biota orientalis, Panax ginseng* and *Schisandra chinensis*. A single dose has been shown to improve memory deficits associated with electro-convulsive shock and the administration of ethanol and scopolamine (Nishiyama *et al,* 1995a). Chronic administration has been shown to ameliorate memory deficits in senescence-accelerated mice (Nishiyama *et al* 1996). Whilst these effects may be as a consequence of the ginseng content, chronic administration of *Biota orientalis* has also been

shown to ameliorate learning deficits as a consequence of basal forebrain-lesions (Nishiyama *et al* 1995b).

***DX-9386***

The medicinal prescription, DX-9386, has long been used in traditional Chinese medicine as a treatment for disordered cognition. It is a combination of *Panax ginseng*, polygala (*Polygala tenuifolia willdenew*), acorus (*Acorus gramineus soland*) and hoelen (*Poria cocos)* (Smigra *et al,* 1995).

Research using rodents has shown that it can ameliorate the learning deficits following thymectomy (Zhang *et al,* 1994); lesioning of the amygdala (Nishiyama *et al,* 1994a); administration of ethanol (Nishiyama *et al* 1994b); and deficits in senescence accelerated mice (Nishiyama *et al,* 1994c).

Zhang *et al* (1994) demonstrated *in vivo* that DX-9386 potentiates LTP formation in the hippocampus of rats. A further study, looking at the effects of the individual constituents, found that this effect was apparent following administration of both hoelen and ginseng, but not the other ingredients (Smigra *et al* 1995).

### 1.6.4 Ayurvedic Medicine

***Brahmi** (Bacopa monniera)*

Brahmi has a recorded medicinal use stretching back to the 6$^{th}$ century AD (Singh and Dhawan, 1982). As well as being an ingredient in the Ayurvedic preparation 'Mentat' it is also utilised by itself as a mild sedative and as a treatment for memory disorders, epilepsy, and insomnia (Tripathi *et al,* 1996). Its putative mnemonic properties are supported by demonstrations of improved rodent learning in a number of situations (Singh and Dhawan, 1982), and significant increases in the memory span, concentration ability, and overall mental performance of 172 human participants with mild to moderate degrees of mental deficiency treated with Brahmi orally for one year, in comparison to 114 in a placebo group (Agrawal *et al,* 1993). Suggestive

support is also offered by placebo-controlled studies showing improvements in memory and cognitive performance following administration of Brahmi combined with *Convolvulus pluricaulis* (Shankhpushpi) (Dubey *et al,* 1994), and *Acorus calamus* (Vacha) (Gupta *et al,* 1994).

Brahmi has also been shown *in vitro* to be a potent dose dependent antioxidant (Tripathi *et al,* 1996), and *in vivo* to have anti-inflammatory actions (Jain *et al,* 1994).

***Mentat** (BR-16A)*

Mentat is an Ayurvedhic medicinal formulation comprising of 26 plant species (including Brahmi) that is widely used in India for its cognition enhancing properties (amongst others). An extensive animal literature attests to its efficacy in the amelioration of cognitive deficits induced in rats by under-nutrition, postnatal environmental impoverishment and hypoxia (Battacharya, 1994), scopolamine (Battacharya, 1995, Kulkarni and Verma, 1992), electro-convulsive shock (Andrade *et al,* 1994; Joseph *et al,* 1994; Vinekar *et al,* 1998; Faruqi *et al,* 1995), and forced endurance performance (Bhardwaj and Sristava, 1995).

Placebo controlled studies have also demonstrated the amelioration of behavioural and cognitive deficits induced by anti-convulsant medication in mentally retarded and epileptic children (Dave *et al,* 1993). Significant improvements in memory span and attentional performance were also shown in 32 healthy young volunteers following 3 months administration (Agrawal *et al,* 1990a). Agrawal *et al* (1990b) also reported improved memory across three distinct age groups of healthy volunteers, again following 3 months administration of Mentat.

***Trasina***

Trasina is an Ayurvedic preparation containing a number of plant-derived constituents *(Withania somnifera, Ocimum sanctum, Eclipta alba, Tinospora cordifolia, Picrorrhiza kurroa)* and a mineral rock exudate from the Himalayas (shilajit). It is traditionally reputed to

ameliorate cognitive and memory deficits associated with a number of illnesses and dementias (Battacharya and Kumar, 1997). Both clinical (Mukherjee and Roy, 1990) and experimental (Battacharya, 1993) evidence indicates a memory enhancing role. Animal studies have also suggested that *Trasina* can alleviate behavioural decrements associated with cholinergic basal forebrain lesions in an animal model of Alzheimer's disease (Battycharya and Kumar, 1997).

***Indian Ginseng** (Withania somnifera)*

*Withania somnifera* is a major component of Trasina, and has been shown to modulate acetylcholinesterase activity and induce increases in cortical muscarinic acetylcholine receptor capacity in rat brains (Schliebs *et al,* 1997). *W. somnifera* administration has also been shown to exert 'adaptogenic' effects in animals exposed to a number of stressors (e.g. Dhuley, 2000; Singh *et al,* 2001). A further *in vivo* study by Battycharya *et al* (2001) suggests that this effect is at least partly due to mitigation of chronic stress-induced oxidative stress in a number of brain regions.

Mishra *et al* (2000) in a review conclude that evidence suggests that *W. somnifera* exerts positive effects on the central nervous system and a number of physiological parameters.

## 1.7 General Conclusions

The foregoing literature review has concentrated on the two main areas of investigation for this thesis – the Oriental herbal remedies *Ginkgo biloba* and *Panax ginseng,* and the prospective European cognition enhancers *Salvia lavandulaefolia* and *Melissa Officinalis.* Brief mention has also been made of a number of single and multiple herbal preparations that fall outside of the scope of the thesis, but which may owe their reputed efficacy to the same, or similar, mechanisms of action as the above.

The evidence with respect to the Oriental herbal remedies is extensive. *Ginkgo biloba enjoys* a large extant literature, which is overwhelmingly positive. Ginseng, on the other hand, while occupying a popularity and perception only rivalled by its Oriental stable-mate, has a literature which is both difficult to interpret and generally ambiguous.

The evidence with regard to both has been criticised. Despite the unequivocally beneficial tenor of the evidence pertaining to *Ginkgo biloba* it has been suggested that research has failed to utilise sufficiently objective psychometric instruments, and in particular computer-based assessment batteries, and that this is a failing that needs to be addressed (Curtis-Prior *et al,* 1999). The same objection could be levelled at the evidence regarding ginseng, but it would tend to be subsumed within a mire of criticisms directed at the variable doses, quality, differential fractionation, and standardisation of extracts employed throughout the literature, and the lack of methodological rigour throughout the overwhelming majority of the 'human' research (Bahrke and Morgan 1994; 2000; Vogler *et al,* 1999). In both cases a well argued call for adequately objective and methodologically rigorous research has been made (Curtis-Prior *et al,* 1999; Vogler *et al,* 1999).

This quite specific scientific 'call to arms' is echoed by a general sentiment towards the complementary and alternative medicines industry, and its attendant research fraternity, which is best reflected in the demand by the House of Lords Select Committee on Science and Technology (Sixth Report, 'Complementary and Alternative Medicine' 2000), not only for a widened evidence base covering all forms of research into herbal remedies, but also for

methodologically rigorous research capable of unequivocally elucidating efficacy (in the case of herbal remedies the committee suggests that this should take the form of randomised controlled trials).

It seems from the above that an adequate starting point for taking research forward in this domain must ideally incorporate three specific criteria: well standardised or defined extracts; objective computer-based measures of cognitive functioning; and rigorous methodological design. It is axiomatic that in the case of the, as yet, untouched research area of the cognitive effects of the European herbs, that this would also provide a necessary starting point, with the proviso that evidence of efficacy from experimentation should drive the development of standardised extracts where none yet exist.

With regard to the first of these criteria - standardisation and definition of extracts - the Oriental herbal products *Ginkgo biloba* GK501 (standardised to 24% flavonoids and 6% terpenoids), and *Panax ginseng* G115 (standardised to 4% ginsenosides), will be utilised. The former (GK501) represents one of the three most widely employed 'gold-standard' *Ginkgo biloba* extracts and is equivalent in quality and active ingredient contents to both Egb761 and LI1370. The latter (G115) is the single most widely taken ginseng extract and, as an example, has been estimated to have enjoyed 50% of the US ginseng market in 1997 (Wilke, 1997). Both have been used extensively in research.

The European herbs are somewhat more problematic. In the initial investigation a standardised, dried, methanol extract of *Melissa Officinalis*, which benefits from rigorous production controls (see Chapter 10), and which is widely marketed in combination with Valeriana, will be utilised. As no recognised extract of *Salvia lavandulaefolia* is currently readily available, it will be necessary to have this extract produced and encapsulated to order. In the case of both extracts the relative lack of definition will be addressed by assessing the theoretically relevant properties of the extracts using *in vitro* analyses.

With regard to the choice of psychometric instruments, the primary tool will be the Cognitive Drug Research (CDR) test battery. This is the only integrated computerized cognitive

assessment system that has thus far been utilized in research into herbal remedies. It has previously been shown to be sensitive to the cognitive effects of *Ginkgo biloba* (Rai *et al,* 1991; Wesnes *et al,* 1987). More recently it has been utilized in an investigation of the cognitive effects of a *Ginkgo biloba/Panax ginseng* combination in impaired (Wesnes *et al.* 1997) and healthy middle-aged cohorts (Wesnes *et al,* 2000). What may make it a particularly useful instrument is recent factor analysis data (see Wesnes *et al,* 2000, or Chapter 2 - Method) that has demonstrated relatively discrete loading of individual task outcome measures onto five cognitive 'factors'. Use of this factor structure, in conjunction with the traditional single task measures, may serve two purposes. Firstly, it may serve to elucidate subtle effects across an entire 'cognitive domain' that would not be detectable with single task data. Secondly, it may facilitate meaningful interpretation in terms of specific cognitive domains.

Secondary assessment instruments will include serial subtraction tasks, which it has previously been suggested are sensitive to augmented delivery of metabolic substrates (Kennedy and Scholey, 2000, Scholey *et al,* 2001), and which will be utilised in a recently developed computerised form (Scholey *et al,* 2001). Concomitant mood, physiological and electrophysiological (EEG) effects of some of the herbal remedies under scrutiny will also be investigated.

With regard to the last criteria, methodology, bare minimum standards of double-blinding, placebo-control, and random allocation to treatment regimes, will be observed in all studies. With regards the general methodological approach of the thesis the question 'where to start?' is necessarily raised. As Curtis-Prior *et al* (1999) point out, even the *Ginkgo biloba* literature has a paucity of studies utilising objective computer-based instruments. Furthermore, ginseng has benefited from the barest minimum of well-controlled research, and the European Labiatiaes have not, as yet, been investigated scientifically in humans. Bearing this in mind it seems appropriate to adopt a '*tabula rasa*' approach.

A first question, therefore, that must be addressed with regard to any putative psychopharmacological interventions that theoretically possess a number of properties that

should be able to modulate cognitive performance (in the absence of long term reorganization of physiological structures or mechanisms), is the acute effects of a single dose. Indeed, in the case of herbal remedies that have never benefited from any single dose studies (curiously, by unsupported convention, the acute effects of ginseng have never been investigated in humans), it would seem necessary to employ multiple single doses in order to identify dose response relationships. Similarly, as no studies have addressed the effects of any of the relevant herbals over a period of time, it would seem necessary to employ multiple testing sessions over a number of hours to identify the time course of any effects.

It is necessary to acknowledge that the double-blind, placebo controlled, randomised, multiple-dose, multiple testing session studies that will make up the majority of this thesis, are not intended to answer all fundamental questions. For instance, the long term, accumulative or protective effects over time of chronic regimens of herbal remedies will not be addressed. Whilst in the long run questions such as this are of paramount importance, foremost because this is liable to be the way in which people will utilise such products, it is necessary to start with the basics and provide an adequate, wide foundation for the concerted scientific research that will be required to address the wider questions.

**Objectives**

The objective of the studies making up this thesis is to investigate the potentially beneficial effects on cognitive performance, and the mechanisms underlying such effects, of acute administration of a number of herbal extracts. The main aims of the present thesis are:

1) To assess the cognitive effects of administration of acute doses of: *Ginkgo biloba; Panax ginseng;* a product combining *Ginkgo biloba* and *Panax ginseng; Melissa officinalis;* and *Salvia lavandulaefolia,* utilising objective computer-based assessment tools, and with specific attention being paid to the time course and dose response relationship of their effects on cognition.
2) To examine concomitant physiological and electrophysiological effects of *Ginkgo biloba* and *Panax ginseng* administration.

# CHAPTER 2. THE COGNITIVE EFFECTS OF ACUTE ADMINISTRATION OF GINKGO BILOBA

## 2.1. Introduction

In humans, the beneficial effects of chronic ginkgo administration have been shown to ameliorate the symptoms of a number of disorders involving both peripheral and central circulatory disturbances (Kleijnen and Knipschild, 1992a). Objectively established improvements in the cognitive decline associated with a number of these disorders have been shown, including demonstrations of benefits in sufferers from intermittent claudication (Draebeck *et al,* 1996), Alzheimer's disease and Vascular dementia (Kanowski *et al,* 1996; Le Bars *et al,* 1997), and a number of generalised conditions with a cerebro-vascular aetiology often encompassed within the umbrella term 'cerebral insufficiency' (Hopfenmüller, 1994; Kleijnen and Knipschild, 1992b). Within this latter group, improvements have been demonstrated relative to placebo in short-term memory and rate of learning following 24 weeks administration of ginkgo (Grässel 1992) and in both reaction times and accuracy throughout a computerised test battery during an 8-week trial (Wesnes *et al,* 1987).

Those studies that have examined the potential of ginkgo as a cognition enhancer in non-pathological populations have tended to concentrate on sufferers from age-associated memory decline. Findings in this domain include increased speed of information processing, as measured by a dual coding task, following a single dose of either 320 mg or 600 mg of the standardised ginkgo extract Egb 761 (Allain *et al,* 1993), and improved performance on the digit copying sub-test of the Kendrick battery and shortened reaction times on a computerised classification task, following 12 and 24 weeks administration of 120 mg of ginkgo extract per day (Rai *et al,* 1991).

The evidence of efficacy in the cognitive enhancement of asymptomatic volunteers includes increased speed on Stroop task, in comparison to placebo, in 'cognitively intact' healthy, older (55-86 years) participants (Mix and Crews, 2000). Several studies have also addressed this

issue in younger cohorts, and share a similar, multiple-dose (of standardised extracts), double-blind, placebo-controlled, balanced-crossover design, with a 5- to 7-day wash-out period between trials. For instance, Rigney *et al,* (1999) examined the effects of 2-day administration regimens of four doses (120-300 mg) of ginkgo. Thirty-one participants, ranging in age from 30 years to 59 years, were administered a battery of tests at baseline and then at hourly intervals over 2 days (1000-2100 hours). In comparison with placebo, performance was only significantly improved on reaction times for the Sternberg short-term numeric memory test on day 1 and day 2 for 120 mg and 300 mg ginkgo extract, and on day 2 alone for 240 mg. These improvements were also more marked for the older participants. These results supported the findings of a previous study by Hindmarch (1986), who utilised three of the eight cognitive tests used in the Rigney study (critical flicker fusion, choice reaction time, Sternberg test). Hindmarch tested eight healthy young participants 1 h after the administration of 120, 240 and 600 mg ginkgo extract and a placebo. Once again, the reaction times on the Sternberg short-term numeric memory test were the only measure significantly improved, and then only in the 600-mg condition. These results have to be viewed in the light of a study by Warot *et al,* (1991) who, in a replication of Hindmarch's study, compared the effects of two 600-mg doses of different ginkgo preparations against placebo on 12 young participants. Warot *et al* included further tasks assessing free and recognition picture recall. They found no difference on the Sternberg short-term numeric memory test, but a significant improvement for one of the ginkgo preparations (Tanakan) on the free picture recall task. Similarly, in a parallel groups study investigating the effects of a sub-chronic 5 day administration of either 120 mg of ginkgo (LI1370) or a placebo to 60 healthy young males Moulton et al (2001) found no interpretable significant differences on any of the tasks employed, which included the Sternberg short term memory test, vocabulary and digit span subtests of the WAIS-R, a reading span test, and a prose recall test.

Whilst evidence is accumulating for a possible role for *Ginkgo biloba* in the attenuation of cognitive deficits due to disease and old age, the direct evidence of a cognition-enhancing role

in younger asymptomatic populations, as outlined above, could be best described as suggestive. It should be noted, however, that previous research has demonstrated improvements in haematological parameters as a consequence of a single dose of ginkgo extract given to healthy young adults (Jung *et al,* 1990), and as a consequence of chronic administration to mountaineers at altitude (Roncin *et al,* 1996). Taken together with evidence of dose-dependent cognitive activation effects of ginkgo on EEG profiles in healthy young volunteers (Itil *et al,* 1996, Luthinger et al 1995), it seems probable that *Ginkgo biloba* may exert a beneficial general effect on cognitive processes in this latter population.

Curtis-Prior et al (1999) note that, with few exceptions, the cognition enhancing effects of ginkgo have been investigated, across populations, with a range of instruments varying from self-report questionnaires to a disparate collection of neuropsychological tasks. This is certainly the case in those studies that have focussed on asymptomatic cohorts. Curtis Prior *et al* (1999) call for the use of more objective systems of assessment, and in particular they advocate the use of computer-based assessment systems in order to provide convincing evidence of *Ginkgo biloba's* efficacy. It seems axiomatic that, as a first step, such objective computer-based research might first be directed towards healthy young populations.

One assessment system that might prove useful in this respect is the Cognitive Drug Research (CDR) Ltd. integrated computerised test battery. This battery has previously been shown to be sensitive to the cognitive effects of both *Ginkgo biloba* (Rai *et al,* 1991; Wesnes *et al,* 1987a) and a *Ginkgo biloba/Panax ginseng* combination in impaired and middle-aged cohorts (Wesnes *et al,* 1997; 2000). One aspect of this battery that might prove particularly useful is the existence of discrete cognitive 'factors', derived by factor analysis (Ward and Wesnes, 1999; Wesnes *et al,* 2000) that has demonstrated relatively discrete loading of individual task outcome measures onto five individual factors. Examination of the individual outcome measures that make up these factors (see Materials and Methods – P 91) suggest that they can usefully be described as corresponding to – accuracy of episodic secondary memory - accuracy of working memory - the speed of memory performance - the speed of attentional task

performance – and the accuracy of attentional task performance. The use of these factors, in concert with individual task outcomes, may serve two purposes. Firstly, subtle treatment related effects across all of the tasks loading on a cognitive domain factor may be apparent despite a lack of significant modulation of individual task outcomes. Secondly, the factor loadings, which fall into intuitively pleasing cognitive domains, may aid interpretation of the overall pattern of treatment effects.

Given the above, the present study was undertaken to investigate the possibility that acute administration of ginkgo may result in cognitive enhancement in healthy young volunteers, with reference to the cognitive domain factors that can be derived from the complete CDR battery.

## 2.2. Materials and Methods

### Participants

Eighteen female and two male undergraduate volunteers (mean age 19.9 years, SD 1.47) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation, each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. Of the 20 participants two were light social smokers and they agreed to abstain from smoking on the day of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Cognitive Measures

The Cognitive Drug Research (CDR) computerised assessment battery (Wesnes, 1987b) has been used in hundreds of European and North American drug trials, and has been shown to be sensitive to acute cognitive improvements (e.g. Moss *et al,* 1998; Scholey *et al,* 1999) as well as impairments with a wide variety of substances (e.g. Ebert *et al,* 1998; O'Neill *et al,* 1995).

A tailored version of the CDR battery was used. This has previously been found to be sensitive to improved cognitive function as a consequence of chronic administration of a *Ginkgo biloba/Panax ginseng* combination (Wesnes *et al,* 1997; Wesnes *et al,* 2000). The selection of computer controlled tasks from the system was administered with parallel forms of the tests being presented at each testing session. Presentation was via VGA colour monitors, and, with the exception of written word recall tests, all responses were recorded via two-button

(YES/NO) response boxes. The entire selection of tasks took approximately 20 minutes. Tests were administered in the following order:

*Word Presentation*: Fifteen words, matched for frequency and concreteness, were presented in sequence on the monitor for the participant to remember. Stimulus duration was 1 second, as was the inter-stimulus interval.

*Immediate Word Recall*: The participant was allowed 60 seconds to write down as many of the words as possible. The task was scored as number correct, errors and intrusions and the resulting score was converted into a percentage.

*Picture Presentation*: A series of 20 photographic images were presented on the monitor at the rate of 1 every 3 seconds, with a stimulus duration of 1 second, for the participant to remember.

*Simple Reaction Time*: The participant was instructed to press the 'YES' response button as quickly as possible every time the word 'YES' was presented on the monitor. Fifty stimuli were presented with an inter-stimulus interval that varied randomly between 1 and 3.5 seconds. Reaction times were recorded in msecs.

*Digit Vigilance Task*: A target digit was randomly selected and constantly displayed to the right of the monitor screen. A series of digits was presented in the centre of the screen at the rate of 80 per minute and the participant was required to press the 'YES' button as quickly as possible every time the digit in the series matched the target digit. The task lasted one minute and there were 15 stimulus-target matches. Task measures were accuracy (%), reaction time (msecs) and number of false alarms.

*Choice Reaction Time*: Either the word 'NO' or the word 'YES' was presented on the monitor and the participant was required to press the corresponding button as quickly as possible. There were 50 trials, of which the stimulus word was chosen randomly with equal probability, with a randomly varying inter-stimulus interval of between 1 and 3.5 seconds. Reaction times (msec) and accuracy (%) were recorded.

*Spatial Working Memory*: A pictorial representation of a house was presented on the screen with four of its nine windows lit. The participant was instructed to memorise the position of the illuminated windows. In 36 subsequent presentations of the house, one of the windows was illuminated and the participant decided whether or not this matched one of the lighted windows in the original presentation. The participant made their response by pressing the 'YES' or 'NO' response button as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages which were used to derive a '% greater than chance performance' score.

*Numeric Working Memory*: Five digits were presented sequentially for the participant to hold in memory. This was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the 'YES' or 'NO' response button as appropriate as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages which were used to derive a '% greater than chance performance' score.

*Delayed Word Recall:* The participant was again given 60 seconds to write down as many of the words as possible. The task was scored as number correct, errors and intrusions and the resulting score was converted into a percentage.

*Delayed Word Recognition*: The original words plus 15 distractor words were presented one at a time in a randomised order. For each word the participant indicated whether or not he recognised it as being included in the original list of words by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages which were used to derive a '% greater than chance performance' score.

*Delayed Picture Recognition*: The original pictures plus 20 distractor pictures were presented one at a time in a randomised order. For each picture participants indicated whether or not it

was recognised as being from the original series by pressing the 'YES' or 'NO' button as appropriate and as quickly as possible. Mean reaction times were measured in msec, and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages which were used to derive a '% greater than chance performance' score.

**Primary cognitive outcome measures**

The above measures were collapsed into a number of cognitive factors derived from the battery by factor analysis. The statistical basis for combining individual task outcome scores into cognitive factors was provided by the factor analysis of performance data from 272 healthy, middle-aged participants (Mean age 56 years, range 38-66 years) taking part in a previous study (Wesnes *et al*, 2000). A principal component analysis was conducted, which detected 5 factors in the data that had eigenvalues greater than unity. These were selected for Varimax rotation. The output from the analysis of the five rotated factors is presented in Table 2.1. These results are also consistent with subsequent factor analyses of the CDR battery outcomes (Ward and Wesnes, 1999; Wesnes *et al*, 2002), and a confirmatory analysis of data from the first baseline session (day 2) of the studies described in chapters 2, 3, 4, 7 and 10 from the current thesis (100 data points per measure). For further details of the factor analysis see Wesnes et al (2000).
The current study utilised the five cognitive factors as primary outcomes on the basis of the factor analysis outlined above. A further 'Quality of Memory' measure was also utilised (see below). The running order of tasks, and the contribution of single task outcomes to the cognitive factors are represented diagrammatically in Figure 2.1.

***Quality of Memory measure:*** is comprised of the single outcome measures making up both the 'Secondary Memory' and 'Working Memory' factors (see below). This global memory measure was included on the basis that it formed the primary outcome in two previous investigations of the cognitive effects of a ginkgo/ginseng combination (Wesnes *et al*, 1997; 2000)

| Factor Name and Individual task measures | Factor 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| **Secondary memory** | | | | | |
| Immediate word recall accuracy | **0.84** | | | | |
| Delayed word recall accuracy | **0.83** | | | | |
| Word recognition accuracy | **0.69** | | | | |
| Picture recognition accuracy | **0.50** | | | | |
| **Working Memory** | | | | | |
| Numeric working memory accuracy | | **0.77** | | | |
| Spatial working memory accuracy | | **0.73** | | | |
| **Speed of Memory** | | | | | |
| Picture recognition speed | | | **0.80** | | |
| Word recognition speed | | | **0.77** | | |
| Numeric working memory speed | | | **0.74** | | |
| Spatial working memory speed | | *-0.37* | **0.67** | | |
| **Speed of Attention** | | | | | |
| Simple reaction time | | | | **0.81** | |
| Choice reaction time | | | *0.39* | **0.68** | |
| Digit vigilance task speed | | | | **0.65** | |
| **Accuracy of Attention** | | | | | |
| Digit vigilance task accuracy | | | | | **0.76** |
| Choice reaction time accuracy | | | | | **0.47** |
| Digit vigilance false alarms | | | | *-0.45* | **-0.53** |

*Table 2.1* Output of principal components analysis showing the Varimax rotated factor structure of the measures that made up the tailored CDR assessment battery utilised in the current study. Significant factor loadings are shown. Italicised factor loadings represent secondary loadings of single measures. These were omitted from the relevant cognitive factor. Adapted from Wesnes *et al* (2000).

***Secondary Memory:*** derived by combining the percentage accuracy scores (adjusted for proportions of novel and new stimuli where appropriate) from all of the secondary memory tests - word recognition, picture recognition, immediate word recall and delayed word recall (with adjustments to the total % correct for errors and intrusions on the latter two tasks). One hundred percent accuracy across the four tasks would generate a maximum score of 400 on this index.

***Working Memory:*** derived by combining the percentage accuracy scores from the two working memory tests - spatial working memory, and numeric working memory. One hundred percent accuracy across the two tasks would generate a maximum score of 200 on this index.

***Speed of Memory:*** derived by combining the reaction times of the four computerised memory tasks - numeric working memory, spatial memory, delayed word recognition, and delayed picture recognition (units are summed milliseconds for the 4 tasks).

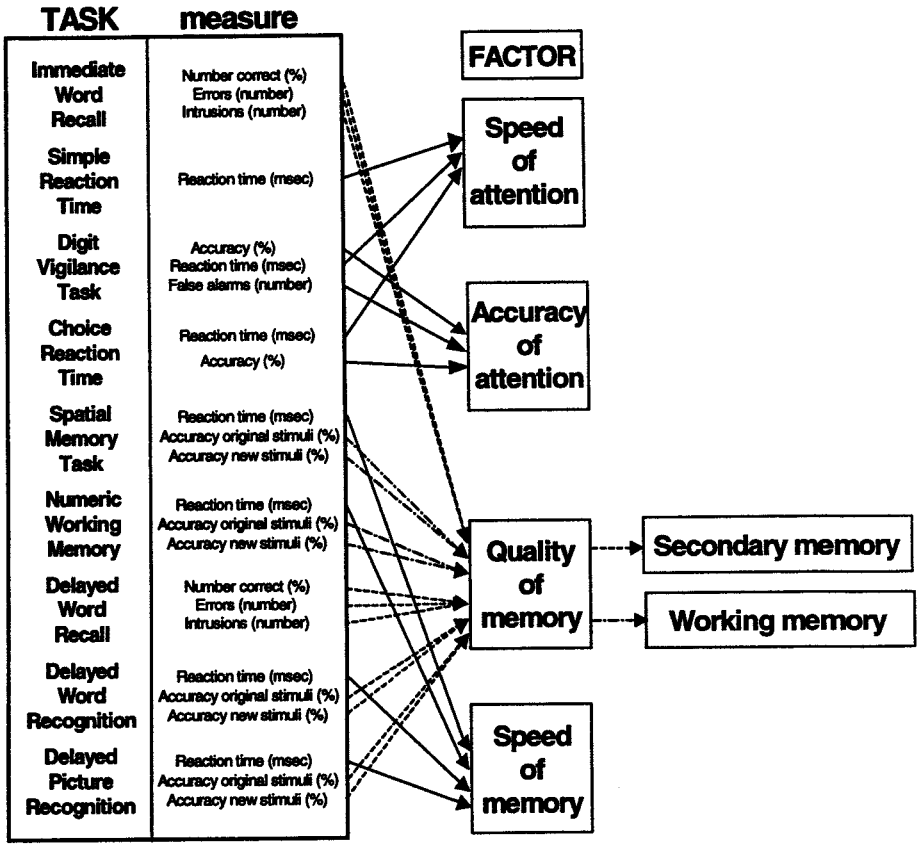


Figure 2.1 running order of individual tasks from the CDR cognitive assessment battery, indicating to which factor the task outcome contributes.

***Speed of Attention***: derived by combining the reaction times of the three attentional tasks - simple reaction time, choice reaction time and digit vigilance (units are summed milliseconds for the 3 tasks).

***Accuracy of Attention:*** derived by calculating the combined percentage accuracy across the choice reaction time and digit vigilance tasks with adjustment for false alarms from the latter test. 100% accuracy across the two tasks would generate a maximum score of 100.

**Subjective mood measure**

The 16 Bond-Lader Visual Analogue Scales (Bond and Lader, 1974 – see Appendix I) were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

**Serial Subtraction tasks**

As part of a theoretically distinct investigation two serial subtraction mental arithmetic tasks were also performed. Experimental rationale, task details and results are reported separately in Chapter 5.

## Treatments

On each study day, participants received six capsules of identical appearance, each containing either an inert placebo or 60 mg of *Ginkgo biloba* extract (GK 501, Pharmaton SA, Lugano, Switzerland) standardised to a content of 24% ginkgo flavone glycosides and 6% terpene lactones. Depending on the condition to which they were allocated on that particular day, the combination corresponded to a dose of either 0 (placebo), 120 mg, 240 mg or 360 mg *Ginkgo biloba* extract.

**Procedure**

Each participant was required to attend a total of five study days that were conducted seven days apart to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session, which established baseline performance for that day, and was immediately followed by the day's treatment on visits 2 to 5. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader visual analogue scales, followed by the CDR test battery. The serial subtraction tasks were completed after these tasks at each testing session.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, and the individual task outcome measures making up the factors, were analysed as 'change from baseline' using the SAS statistical package. The initial analysis was made using the general linear models procedure (PROC GLM). Following the recommendations of Keppel (1991) the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three *Ginkgo biloba*

conditions (120 mg, 240 mg, 360 mg)) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

The three mood outcomes derived from the Bond-Lader scales were analysed using a within subjects Analyses of Variance (Minitab) with planned comparisons as per the above.

The statistical approach adopted here is discussed further in Chapter 13 (Discussion - section 13.3).

## 2.3. Results

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 120mg, 240mg, and 360mg of GK501) for the cognitive factor scores, and Bond-Lader mood scale scores were subjected to a one-way, repeated-measures Analysis of Variance. There were no significant differences in baseline performance on any of these measures.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are presented in Table.2.2. Significant results on individual task outcomes are presented in relationship to the overall factor to which they contribute below (memory task results are presented with the relevant factor i.e. 'Secondary' or 'Working' memory).

### Cognitive factor outcome measures

Mean raw and change from baseline cognitive factor outcome measure scores for each condition across each session are displayed in Figure. 2.2.

#### *Quality of Memory measure*

Planned comparisons revealed significant improvements, compared with placebo, for 120 mg of ginkgo at both 1 hour [$t$ (171) =2.14; p=0.033] and 4 hours [$t$ (171)=2.32; p=0.02] post-dose. A similar pattern was evinced for 240 mg of ginkgo, with trends towards an improvement in comparison with placebo at the same time points (1 h post [$t$ (171)=1.82; p=0.069], 4 h post [$t$ (171)=1.81; p=0.071]. There were no significant improvements associated with the 360-mg dose of ginkgo.

***Secondary Memory Factor***

Differences in change from baseline performance on the 'Secondary Memory' factor reached significance for the 240mg dose alone at 4 hours post dose [*t* (171)=2.19; p=0.03). However there were strong trends towards improved performance for the 120mg dose at both 1 hour [*t* (171)=1.88, p=0.060] and 6 hours post-dose [*t* (171)=1.95; p=0.053).

Inspection of the single measures that contribute to the 'Secondary Memory' factor showed that the only task that evinced a coherent pattern of modulated performance was the delayed word recall task, with significant improvements for 120mg and 240mg of ginkgo at 1 hour post-dose ([*t* (171)=2.27, p=0.024] and [*t* (171)=2.2, p=0.029] respectively), and for 240mg and 360mg at 2.5 hours post-dose ([*t* (171)=2.05, p=0.04] and [*t* (171)=2.6, p=0.01] respectively). Two of the other three component task measures exhibited a single significant difference in performance with an improvement seen on the delayed word recognition task at 4 hours following 240mg of *ginkgo* [*t* (171)=2.38, p=0.018], and a significant decrement in performance on the immediate word recall task for the same dose at 6 hours [*t* (171)= 2.78, p=0.006]. Results for the delayed picture recognition task showed both a single improvement in performance (120mg at 4 hours [*t* (171)= 3.12, p=0.002]) and a single decrement in performance (260 mg at 2.5 hours [*t* (171)= 2.56, p=0.011]. Given the statistical method utilised, the interpretation of single significant differences, as seen on the latter three measures, would be unwise.

***Working Memory Sub-Factor***

Whilst there were no significant differences on this factor, reference to the single task outcomes shows that the highest dose of ginkgo (360 mg) under performed the placebo group on the numeric working memory task at all time points, with this effect reaching significance at both 1 hour [*t* (171)= 2.18, p=0.031] and 6 hours post-dose [*t* (171)= 2.71, p=0.007]. The spatial memory task was unaffected.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Immediate Word Recall (% accuracy) | placebo | 55.83 3.98 | -7.50 2.99 | -5.83 4.38 | -7.00 3.33 | -1.83 3.04 |
| | 120mg | 50.67 4.07 | -1.83 3.19 | -6.00 3.39 | -1.83 3.66 | -8.50 3.50 |
| | 240mg | 54.50 3.67 | -5.33 3.41 | -5.50 3.56 | -1.83 3.61 | -12.17 2.93** |
| | 360mg | 55.67 3.57 | -5.17 3.36 | -5.83 3.74 | -8.50 3.69 | -9.00 4.31 |
| Simple Reaction time (msecs) | placebo | 257.65 6.40 | 10.80 5.77 | 11.37 5.59 | 14.89 6.47 | 20.20 7.09 |
| | 120mg | 256.94 5.23 | 15.68 5.85 | 18.62 5.94 | 7.09 5.46 | 17.70 7.40 |
| | 240mg | 257.17 4.82 | 7.52 3.47 | 16.31 5.55 | 13.11 6.10 | 23.85 5.51 |
| | 360mg | 264.61 5.89 | 3.67 4.86 | -1.06 3.91* | 10.87 5.67 | 5.32 7.01* |
| Digit Vigilance Accuracy (%) | placebo | 97.33 1.32 | -0.33 1.71 | -3.67 1.71 | -1.33 1.97 | -0.67 1.07 |
| | 120mg | 96.00 1.12 | -1.00 2.12 | 0.67 1.67** | 0.33 1.41 | -1.00 1.47 |
| | 240mg | 97.67 1.00 | -1.00 1.62 | -2.33 1.83 | -2.00 1.61 | -0.33 1.41 |
| | 360mg | 97.00 1.02 | 0.33 1.23 | 0.67 1.07** | 0.00 1.45 | -0.67 1.27 |
| Digit Vigilance False alarms (number) | placebo | 0.15 0.08 | 0.10 0.12 | 0.20 0.19 | 0.25 0.27 | 0.20 0.14 |
| | 120mg | 0.45 0.15 | 0.35 0.21 | 0.15 0.15 | -0.05 0.15 | 0.25 0.29 |
| | 240mg | 0.25 0.12 | 0.15 0.18 | 0.10 0.16 | 0.30 0.29 | 0.25 0.20 |
| | 360mg | 0.55 0.15 | -0.25 0.19 | -0.10 0.19 | 0.00 0.32 | -0.15 0.20 |
| Digit Vigilance Reaction time (msecs) | placebo | 382.75 9.00 | 13.40 5.67 | 24.06 7.32 | 21.53 9.27 | 30.97 7.98 |
| | 120mg | 392.00 6.14 | 20.68 8.10 | 18.85 8.23 | 16.17 7.94 | 31.40 8.70 |
| | 240mg | 398.02 8.10 | -4.51 8.88* | 7.09 7.06* | 8.60 7.29 | 8.41 7.79** |
| | 360mg | 393.51 7.26 | 12.92 6.45 | -8.20 6.48*** | 5.22 9.32* | 25.91 6.67 |
| Choice reaction time accuracy (%) | placebo | 94.40 0.73 | 1.20 0.72 | 1.00 0.72 | -0.50 1.06 | 0.10 0.96 |
| | 120mg | 95.30 0.76 | -0.80 0.59 | -0.10 0.91 | -0.30 0.64 | -1.10 0.70 |
| | 240mg | 96.90 0.51 | -1.60 0.57** | -2.20 0.90**** | -1.60 0.69 | -2.50 0.72*** |
| | 360mg | 96.60 0.63 | -1.60 1.17** | -1.70 0.97*** | -0.80 0.72 | -2.40 1.11** |
| Choice reactionTime (msecs) | placebo | 394.72 9.21 | 6.23 7.51 | 19.89 11.54 | 21.90 9.13 | 14.19 10.60 |
| | 120mg | 399.36 11.39 | 14.64 14.01 | 14.23 9.18 | 8.31 10.07 | 10.19 8.20 |
| | 240mg | 398.68 9.61 | 5.68 6.32 | 2.03 8.54* | 4.35 10.52* | 1.18 8.58 |
| | 360mg | 412.76 10.37 | 1.78 12.45 | -10.33 8.58**** | -12.61 7.42***** | -16.62 7.49***** |
| Spatial Memory (%>chance) | placebo | 92.19 1.54 | -8.38 4.30 | -2.81 2.77 | -8.00 6.32 | -8.63 3.67 |
| | 120mg | 88.44 3.32 | -4.56 4.39 | -5.06 5.30 | 2.56 3.61* | 1.13 3.96 |
| | 240mg | 94.94 1.30 | -4.13 2.25 | -6.88 3.18 | -4.50 2.00 | -3.00 2.09 |
| | 360mg | 93.81 1.24 | -11.94 7.47 | -2.50 1.94 | -7.38 3.80 | -8.19 5.79 |
| Spatial memory Reaction time (msecs) | placebo | 555.77 26.80 | -6.23 18.47 | -7.97 14.56 | -17.81 14.52 | -31.71 18.41 |
| | 120mg | 540.90 20.27 | -5.42 19.65 | 19.46 32.38 | -1.97 12.62 | -13.54 15.22 |
| | 240mg | 549.83 21.18 | -7.56 11.07 | 0.85 14.21 | 6.03 14.90 | -9.59 23.39 |
| | 360mg | 549.73 25.85 | 9.39 29.30 | -37.94 18.13 | 5.43 38.77 | -36.17 14.27 |
| NumericWork'g Memory (%>chance) | placebo | 85.33 2.40 | -2.56 2.53 | -2.67 1.86 | -3.22 2.81 | -1.00 1.90 |
| | 120mg | 86.00 2.27 | 0.44 2.36 | -1.33 2.97 | -5.33 3.38 | -2.45 2.36 |
| | 240mg | 88.67 2.09 | -0.89 2.23 | -1.22 1.52 | -7.89 3.15 | -3.00 2.34 |
| | 360mg | 89.89 1.78 | -8.00 2.91* | -2.78 1.79 | -6.11 1.54 | -7.78 2.65** |
| Numeric Working Memory Reaction Time (msecs) | placebo | 550.15 21.19 | -16.81 13.11 | -8.65 14.34 | -31.33 11.29 | -18.14 16.70 |
| | 120mg | 568.12 24.10 | -41.10 12.94 | -32.52 12.34 | -27.71 14.57 | -31.24 13.47 |
| | 240mg | 545.75 18.84 | -5.24 12.23 | -16.25 13.29 | -10.15 15.23 | -26.14 12.01 |
| | 360mg | 543.56 22.26 | -26.86 21.43 | -20.84 20.34 | -3.46 10.12* | -43.15 20.18 |
| Delayed Word Recall (% accuracy) | placebo | 46.50 4.73 | -19.50 3.65 | -22.00 4.11 | -16.33 5.13 | -20.00 3.77 |
| | 120mg | 40.33 4.56 | -9.17 3.94* | -16.67 3.97 | -17.00 4.64 | -22.67 3.10 |
| | 240mg | 42.33 4.12 | -13.00 4.31 | -12.67 4.30* | -13.50 4.64 | -15.50 5.15 |
| | 360mg | 42.17 4.33 | -9.50 3.72* | -10.17 3.54* | -18.33 3.99 | -17.17 4.62 |
| Word Recognition (%>chance) | placebo | 68.67 4.86 | -7.92 4.14 | -8.33 5.45 | -16.00 6.01 | -9.67 4.52 |
| | 120mg | 64.33 4.57 | -5.94 3.71 | -10.00 5.24 | -12.00 4.50 | -17.33 4.85 |
| | 240mg | 65.04 3.58 | -3.71 3.29 | -6.71 2.60 | -4.71 3.39* | -12.04 4.90 |
| | 360mg | 66.33 3.30 | -4.33 2.48 | -6.33 3.73 | -10.00 5.04 | -5.23 5.44 |
| Word Recognition Reaction time (msecs) | placebo | 628.09 23.26 | 22.39 17.02 | 26.21 12.26 | 8.91 16.01 | 44.49 37.52 |
| | 120mg | 646.87 22.79 | 12.54 17.25 | 9.64 20.02 | 9.27 21.12 | -5.75 23.70* |
| | 240mg | 626.54 24.06 | 15.77 20.60 | 19.65 16.78 | 46.35 23.17 | -18.91 12.88** |
| | 360mg | 641.71 21.64 | 6.13 15.73 | -17.89 16.39 | 14.44 18.78 | -21.29 22.25** |
| Picture Recognition (%>chance) | placebo | 66.50 4.50 | -8.50 6.51 | -8.75 3.80 | -19.50 4.31 | -9.00 5.93 |
| | 120mg | 66.75 4.39 | -4.75 5.39 | -10.50 3.48 | -5.50 3.66** | -10.50 4.18 |
| | 240mg | 71.50 5.03 | -3.00 4.27 | -8.50 3.59 | -13.50 4.52 | -11.75 4.12 |
| | 360mg | 74.50 4.15 | -16.00 5.72 | -20.25 6.36* | -15.50 4.56 | -11.75 4.83 |
| Picture recognition Reaction time (msecs) | placebo | 697.49 19.14 | 40.36 19.99 | 19.19 17.13 | 12.11 17.62 | 15.74 24.38 |
| | 120mg | 724.68 30.43 | -14.23 18.49** | -23.25 18.09* | -30.54 24.50* | -36.76 27.09* |
| | 240mg | 702.32 20.43 | 10.99 17.86 | 38.94 20.07 | 45.10 26.10 | 5.10 22.72 |
| | 360mg | 709.50 16.99 | -2.71 19.81* | -4.82 22.58 | -27.46 15.93 | 8.87 19.28 |

*Table 2.2 Effects of Ginkgo biloba (GK501) on individual task outcome measures from the CDR battery. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, ****,=0.001,*****, = 0.0005 compared to placebo).*

*Speed of Memory factor*

Speed was significantly enhanced across the timed memory tasks for 360 mg of ginkgo at 2.5 h post-dose [$t$ (171)=2.07; p=0.04), with trends towards enhancement for both 120 mg and 360 mg at 6 h post-dose ([$t$ (171)=1.83; p=0.068] and [$t$ (171)=1.91; p=0.057] respectively). The 240-mg dose of ginkgo, however, under-performed the other doses at all time points and evinced a significant reduction in speed in comparison with placebo at 4 h post-dose [$t$ (171)=2.16; p=0.03].

Whilst these results are somewhat ambiguous, reference to the single task measures reveals significant speeding of performance on the two timed 'Secondary Memory' tasks. Reaction times on the delayed picture recognition task were significantly improved for the lowest (120mg) dose at all time points (1 hour [$t$ (171)=2.61; p=0.0097], 2.5 hours [$t$ (171)=2.03; p=0.044], 4 hours [$t$ (171)=2.04; p=0.043], and 6 hours post-dose [$t$ (171)=2.51; p=0.013]), and at a single time point (1 hour) for the highest dose (360 mg) [$t$ (171)=2.06; p=0.004]. Speed was also improved for all three doses at the 6 hour testing session on the delayed word recognition task (120 mg [$t$ (171)=2.05; p=0.004], 240 mg [$t$ (171)=2.59; p=0.01], 360 mg [$t$ (171)=2.69; p=0.008]. This evidence of faster performance did not hold true for the 'Working Memory' tasks, with no differences in the speed of performing the spatial memory task and a single significant decrement on the numeric working memory task for the 360 mg dose at 4 hours [$t$ (171)=2.12; p=0.035].

*Quality of attention factor*

There was a single significant decrement in accuracy on the attention tasks, which was restricted to the 240-mg dose at 1 h post-dose [$t$ (171)=2.02; p=0.045]. However, on the single task outcomes there were significant decrements in change from baseline performance on the accuracy of the choice reaction time task which were evinced by both the 240 mg dose (1 hour [$t$ (171)=3.16; p=0.002], 2.5 hours [$t$ (171)=3.61; p=0.0004], 6 hours [$t$ (171)=2.93; p=0.004]) and 360 mg doses of ginkgo (1 hour [$t$ (171)=3.16; p=0.002], 2.5 hours [$t$ (171)=3.04;

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Quality of Memory (%X6) | placebo | 415.02 14.71 | -54.35 12.85 | -50.40 13.76 | -70.06 16.59 | -50.12 11.59 |
| | 120mg | 396.52 15.57 | -25.81 10.07 | -49.56 11.07 | -39.10 10.40 | -60.32 9.84 |
| | 240mg | 416.98 13.27 | -30.05 10.57 | -41.47 10.13 | -45.93 12.25 | -57.46 12.23 |
| | 360mg | 422.37 13.21 | -54.94 10.21 | -47.86 10.93 | -65.82 11.77 | -59.11 13.83 |
| Secondary Memory (%X4) | placebo | 237.50 13.48 | -43.42 12.68 | -44.92 12.95 | -58.83 14.27 | -40.50 12.72 |
| | 120mg | 222.08 14.17 | -21.69 7.69 | -43.17 9.49 | -36.33 9.02 | -59.00 8.09 |
| | 240mg | 233.37 12.85 | -25.04 10.46 | -33.38 8.78 | -33.54 11.36 | -51.46 10.81 |
| | 360mg | 238.67 12.25 | -35.00 8.07 | -42.58 11.02 | -52.33 10.28 | -43.15 11.64 |
| Working Memory (%X2) | placebo | 177.52 3.32 | -10.93 4.20 | -5.48 3.98 | -11.22 7.07 | -9.62 4.24 |
| | 120mg | 174.44 4.78 | -4.12 6.16 | -6.40 5.31 | -2.77 4.78 | -1.32 4.80 |
| | 240mg | 183.61 2.42 | -5.01 3.18 | -8.10 3.99 | -12.39 4.47 | -6.00 3.37 |
| | 360mg | 183.70 2.27 | -19.94 7.61 | -5.28 2.73 | -13.49 3.69 | -15.97 6.26 |
| Speed of Memory (summed msecs) | placebo | 2431.50 73.89 | 39.71 43.42 | 28.77 31.03 | -28.13 31.22 | 10.38 67.08 |
| | 120mg | 2480.58 79.71 | -48.21 39.74 | -26.67 50.92 | -50.94 50.47 | -87.29 46.42 |
| | 240mg | 2424.44 69.75 | 13.97 39.31 | 43.20 45.70 | 87.33 60.34 | -49.54 38.30 |
| | 360mg | 2444.50 70.31 | -14.05 65.53 | -81.50 63.81 | -11.06 53.79 | -91.74 45.05 |
| Quality of Attention (%) | placebo | 90.85 0.73 | 0.35 0.94 | -1.35 0.86 | -1.10 1.32 | -0.45 0.82 |
| | 120mg | 90.40 0.86 | -1.20 1.23 | 0.10 1.01 | 0.05 0.70 | -1.25 0.85 |
| | 240mg | 92.15 0.60 | -1.40 0.89 | -2.25 0.84 | -2.00 0.93 | -1.65 0.88 |
| | 360mg | 91.40 0.58 | -0.40 0.69 | -0.45 0.55 | -0.40 0.88 | -1.35 0.75 |
| Speed of Attention (summed msecs) | placebo | 1035.13 19.17 | 30.44 12.48 | 55.32 12.38 | 58.32 12.87 | 65.36 14.08 |
| | 120mg | 1048.29 18.17 | 51.00 22.40 | 51.69 16.83 | 31.56 16.43 | 59.29 16.89 |
| | 240mg | 1053.86 19.36 | 8.68 14.15 | 25.43 17.84 | 26.07 19.38 | 33.44 15.41 |
| | 360mg | 1070.88 20.01 | 18.37 15.44 | -19.58 14.16 | 3.48 16.06 | 14.60 14.92 |

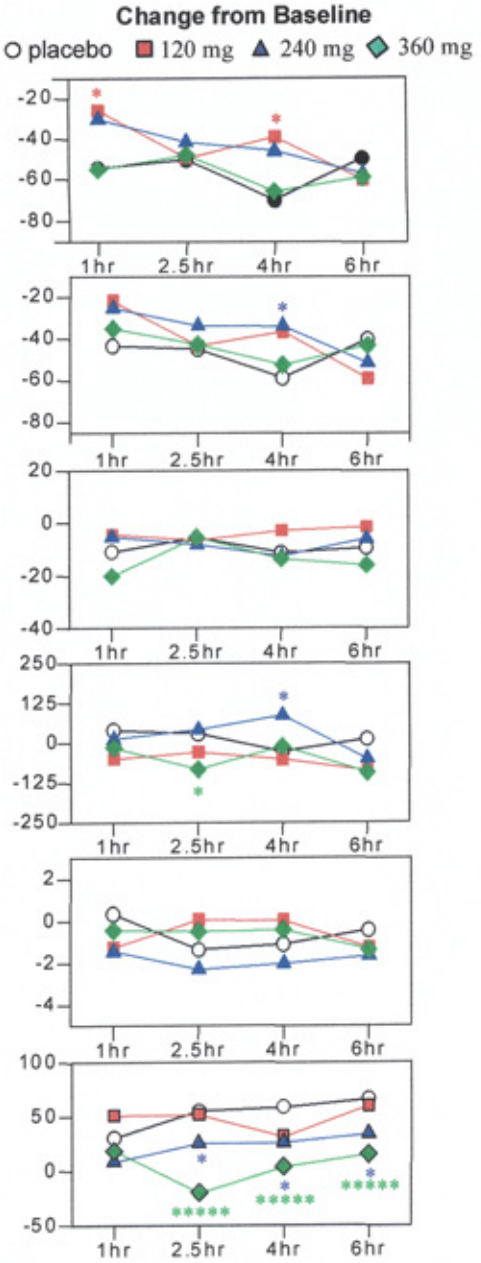


*Figure 2.1.* Effects of *Ginkgo biloba (GK501)* on cognitive measures; , 'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Quality of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of *Ginkgo*. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01,***, p = 0.005; ****, p = 0.001; *****, p = 0.0005 compared to the corresponding placebo score).Units are as per the table.

p=0.003], 6 hours [*t* (171)=2.82; p=0.005]). In contrast, both 120 mg and 360 mg were associated with improved accuracy on the digit vigilance task at 2.5 hours post dose ([*t* (171)=2.9; p=0.004].

***Speed of attention factor***

Speed was significantly enhanced on the attention tasks for 240 mg at 2.5 h [*t* (171)=2.11; p=0.036], 4 h [*t* (171)=2.28; p=0.024] and 6 h post-dose [*t* (171)=2.25; p=0.026]. The same pattern was evident for the 360-mg dose, with enhancement at 2.5 h [*t* (171)=5.28; p=0.0001], 4 h [*t* (171)=3.87; p=0.0002] and 6 h post-dose [*t* (171)=3.58; p=0.0004]. Whilst there were no significant improvements on this factor for the lowest dose (120 mg), it should be noted that there was a trend towards improvement at 4 h [*t* (171)=1.88; p=0.06].

This pattern of results was reflected in all three of the component measures, with the most modest modulation evident for the simple reaction time task with improvements in speed evident only for the 360 mg dose at 2.5 hours [*t* (171)=1.99; p=0.048], and 6 hours post-dose [*t* (171)=2.38; p=0.018]. Improvements were evident on the digit vigilance task reaction times for the 240 mg dose at 1 hour [*t* (171)=2.18; p=0.031], 2.5 hours [*t* (171)=2.06; p=0.04] and 6 hours post-dose [*t* (171)=2.74; p=0.007], and for the 360 mg dose of ginkgo at 2.5 hours [*t* (171)=3.92; p=0.0001] and 4 hours post-dose [*t* (171)=1.98; p=0.049]. Similarly, choice reaction times were improved for the 240 mg dose at 2.5 hours [*t* (171)=2.21; p=0.029] and 4 hours post dose [*t* (171)=2.17; p=0.032], and for the 360 mg dose at 2.5 hours [*t* (171)=3.73; p=0.0003], 4 hours [*t* (171)=4.26; p=0.00003] and 6 hours post-dose [*t* (171)=3.8; p=0.0002].

**Subjective mood measures**

None of the three factors derived from the Bond-Lader visual analogue scales (Bond and Lader 1974) showed a significant difference as a consequence of administration of ginkgo.

## 2.4. Discussion

These results show that acute *Ginkgo biloba* administration can enhance cognitive performance in healthy young adults. This cognition enhancement following the administration of the GK 501 extract was manifested most notably in increased speed of performance on tasks making up the 'Speed of Attention' factor. This effect was both dose and time dependent, with significant improvements seen only for the two highest doses (240 mg and 360 mg) at the later time points, with increased speed on this factor at 2.5, 4 and 6 h following ingestion. The results from individual tasks making up the factor showed increases in speed across all three tasks (simple and choice reaction time and digit vigilance tasks) for the 360 mg dose and across two tasks for the 240 mg dose. It should, however, be noted that accuracy on the choice reaction time task was impaired for both doses at 1, 2.5 and 6 hours post-dose. Whilst this suggests the possibility of a speed-accuracy trade-off it is interesting to note that accuracy on the digit vigilance task showed no decrements, and was significantly improved at one time point for the highest dose.

The only other measure that evinced a convincing pattern of effects was the global 'Quality of Memory' measure (comprised of both 'Working' and 'Secondary' memory factors), on which performance was significantly enhanced for the lowest dose (120 mg) at 1 hour and 4 hours. Whilst the 120 mg dose was only associated with trends towards an improvement on the 'Secondary Memory' factor at both the 1 hour and 4 hour testing sessions, the middle (240 mg) dose evinced a significant enhancement at the 4 hour testing session. However, this has to be viewed in light of reduced speed on the 'Speed of Memory' factor for the same dose at the same time point. It is notable that all three doses evinced isolated improvements on at least two of the four secondary memory tasks. The 'Working Memory' factor was unaffected by any dose, with the only modulation reflecting disturbed performance on the numeric working memory task.

The differing patterns of results on the 'Speed of Attention' and 'Quality of Memory' factors is particularly interesting as the memory enhancement would seem to be manifested for the lowest

dose at the earlier time points, whereas the speed of performance of attention tasks is increased for the highest doses at the later times post-dose. Whether these results represent the working of two distinct pharmacological mechanisms, rather than the separate time- and dose-dependent effects of one mechanism, remains to be elucidated.

Several other significant changes noted across the 'Speed of Memory' and 'Accuracy of Attention' measures, contradictory significant results in the case of the former, and a solitary decrement in accuracy in the case of the latter, all restricted to single time/dose points, are not readily interpretable, particularly given the statistical approach employed.

The findings of the current study offer some support to those of Hindmarch (1986) and Rigney *et al,* (1999), in as much as these studies both found improvements, relative to placebo, restricted to reaction times. In both cases these improvements were seen on the Sternberg short-term numeric memory test. The results here, however, would seem to suggest that the enhancement evinced in the former studies may be attributable to improved speed of performance on the task *per se*. The above authors' suggestions that the cognition-enhancing effect of *Ginkgo biloba* is more pronounced for memory processes receives little support from the current study's finding of comparatively limited improvements on the global memory measures and individual secondary memory tasks utilised here. Indeed, results on the numeric working memory task, which is analogous to the Sternberg task, show limited modulation, restricted to decrements in both accuracy (1 and 6 hours) and speed of performance (4 hours) for the highest dose of ginkgo (360 mg) in comparison to placebo.

The results are largely in line with those from studies investigating the effects of *Ginkgo biloba* in pathological cohorts that have included measures of reaction times. Examples include: an improvement in the combined reaction times derived from a selection of tasks from the CDR battery throughout a 3-month trial involving elderly sufferers from 'idiopathic cognitive impairment' (Wesnes *et al, 1987*a); improvements in simple reaction times (Gessner *et al, 1985*); reaction times on a computerised classification task (Rai *et al, 1991*); and shorter stimulus evaluation times as evinced by decreases in P300 event related potentials following

both acute and chronic administration of ginkgo in sufferers from age-related memory impairment (Semlitsch *et al, 1995*). It is interesting to note that evidence of the other cognitive improvements demonstrated in pathological populations was limited in the current study to relatively mild enhancement of mnemonic function. It seems plausible to suggest that such improvements may reflect an attenuation of age- or disease-related deficits, and would be unlikely to be manifested to any great extent in a young healthy cohort.

Whilst the results of the current study could not be said to constitute an adequate platform for a discussion of possible mechanisms, it is interesting to note similarities with other research findings. Animal experimentation has demonstrated a partial ginkgo related re-establishment of glucose consumption and an increase in glucose transfer rate during hypoxia (Rapin *et al, 1986*). Similarly, prolonged survival time under lethal hypoxia in mice, retardation of the breakdown of brain energy metabolism, and increased local cerebral blood flow in rats, following administration of ginkgo extract, have been demonstrated (Oberpichler *et al, 1988*). In healthy young volunteers, Schaffler and Reeh *(1985)* demonstrated improvements, in comparison with placebo, in complex choice reaction times during hypoxic hypoxia following 14 days treatment with ginkgo. Conversely, utilising the same battery as the current study in an investigation of the cognitive effects of oxygen administration, Moss *et al* (1998) demonstrated dose-dependent improvements on each of the three components of the speed of attention factor (simple, choice, and digit vigilance reaction times), with more restricted improvements observed on the components of the memory factors used here. Evidence suggests that *Ginkgo biloba* can modulate several aspects of the cholinergic system (Nathan, 2000), and it is interesting to note that improvements in secondary memory and attention performance would be predicted from the previous literature on cholinergic modulation of cognitive function (Blokland, 1996; Feldman *et al*, 1997; Rusted and Warburton, 1989). However, it seems equally plausible to suggest that ginkgo might owe its cognition-enhancing effect either to modulation of cerebral cellular metabolism or, alternatively, to improved haemorrheological parameters (Jung *et al*, 1990; Roncin *et al*, 1996), leading to simple augmentation of the levels

of metabolic substrates reaching the brain. In light of research indicating a relationship between the efficiency of glucose delivery and utilisation, and performance on 'demanding' non-memory tasks (Kennedy and Scholey, 2000), it is of interest to investigate *Ginkgo biloba*'s possible role during cognitive demand. It should also be noted that whilst the current study demonstrated improved performance on both the 'Speed of Attention' factor and 'Quality of Memory' measure, two previous studies on the cognitive effects of a *Ginkgo biloba*/*Panax ginseng* combination (Gincosan, Pharmaton SA), which utilised the same factors from the same battery, reported improved performance for sufferers from 'neurasthenic' complaints and the healthy middle aged. Statistically significant improvements were, however, restricted to the 'Quality of Memory' measure (Wesnes *et al,* 1997; 2000).

The findings of the current study are of particular interest as they demonstrate specific pharmacological actions as a consequence of single doses of *Ginkgo biloba*, with this effect being apparent in a population of healthy young participants who could be conceived of as performing near the zenith of their cognitive capabilities.

# CHAPTER 3. THE COGNITIVE AND MOOD EFFECTS OF ACUTE ADMINISTRATION OF *PANAX GINSENG*

## 3.1. Introduction

A wealth of research utilising a variety of species and fractions of ginseng has established a number of possible mechanisms that may contribute to its putative efficacy. These include: effects on both blood pressure (e.g. Wood *et al,* 1964) and vasoconstriction. (e.g. Lee *et al,* 1981; Lei and Chiou, 1986); a beneficial influence on blood flow through modulation of platelet aggregation (e.g. Jung *et al,* 1998; Shi *et al,* 1990); roles in both cardio-protection (e.g. Chen, 1996; Gillis, 1997; Zhan *et al,* 1994) and neuroprotection following a number of insults (e.g. Chen *et al,* 1997; Choi *et al,* 1996; Wen *et al,* 1996); shifting the hormonal balance of the hypothalamic-pituitary-adrenal system (e.g. Filaretov, 1988; Kim *et al,* 1998a; Sonnenborn and Proppert, 1991); and modulation of a number of neurotransmitter systems (e.g. Benishin, 1992; Kim *et al,* 1998b; Petkov, 1978).

Taken alone, the wealth of research, both *in vitro* and *in vivo,* demonstrating a plethora of both microscopic and macroscopic physiological consequences of ginseng administration, would seem to suggest that there should be behavioural consequences to the ingestion of ginseng. To a certain extent this is borne out in the animal literature, with a number of behavioural changes demonstrated in animals following ginseng administration. These include: amelioration of indices of experimentally induced stress (Kim *et a,* 1998a; Nguyen *et al,* 1995); attenuation of fatigue due to forced exercise (Filaretov, 1988; Wang and Lee, 1998) with concomitant improvements in physiological parameters (Fernando *et al,* 1999a; 1999b); attenuation of learning deficits due both to age (Nitta *et al,* 1995) and experimentally induced brain damage (Wen *et al,* 1996; Zhao and McDaniel, 1998); and dose-dependent improvements in learning and memory in both young and old rodents (Petkov *et al,* 1993; Petkov and Mosharrof, 1987).

Regarding humans, a number of studies have investigated the effects of chronic administration of ginseng, either alone or in combination with vitamins. A number of studies have investigated ginseng's effects on physical performance and relevant physiological parameters, but the evidence of efficacy here could best be described as equivocal (Bahrke and Morgan, 1994; 2000). A number of studies have also assessed ginseng's reputed beneficial effect on general well-being, and several of these studies have reported improvements in subjective ratings of 'well being' or 'quality of life' (Marasco *et al,* 1996; Neri *et al,* 1995; Sotaniemi *et al,* 1995; Ussher *et al,* 1995; Wiklund *et al,* 1994), whilst others have not (Hallstrom *et al,* 1982;Thommessen and Laake, 1996). However, several of these studies have also included a cognitive assessment. These have demonstrated enhanced performance, in comparison to placebo, on various cognitive measures. Such improvements include faster completion of a timed diagram drawing test in non-insulin-dependent diabetics (Sotaniemi *et al,* 1995), faster reaction times in older participants (Forgo *et al,* 1981), improved performance on a tapping test for fatigued night nurses (Hallstrom *et al,* 1982), and better performance on the Randt Memory Test in a cohort suffering age-related memory impairment (Neri *et al,* 1995).

Two investigations have focussed directly on ginseng's effects on cognition, both employing a double-blind, placebo-controlled design. D'Angelo *et al* (1986) examined the effects of 12 weeks daily administration of 200 mg of ginseng to healthy young volunteers. Tests included those assessing motor performance, speed of performance on attentional tasks, mental arithmetic and logical reasoning. The result showed that performance was significantly improved for the ginseng group, in comparison to placebo, only on the mental arithmetic test. A later study by Sorensen and Sonne (1996), examined the effects of 400 mg of ginseng daily for 9 weeks in a cohort of 40 to 70 year olds, utilising a slightly more comprehensive battery. There were statistically significant performance improvements for the ginseng group, in comparison to placebo, only on the fastest trials of an auditory simple reaction time test, and on a computerised version of the Wisconsin Card Sort Test.

In summary, despite a huge extant literature dealing with the *in vitro, in vivo, ex vivo* and animal behavioural effects of ginseng, the evidence of beneficial effects in humans tends to be equivocal (Bahrke and Morgan, 1994; 2000; Vogler *et al,* 1999). To a great extent this lack of firm support for efficacy in humans can be accounted for by methodological considerations. The first of these concerns the different doses, types and fractions of ginseng utilised, the frequent inclusion of adulterants, and a general lack of adequate standardisation in the administered extracts. The second area concerns variations in the rigour of experimental designs. As an example, Bahrke and Morgan (1994) note that human ergogenic research is typified by an absence of appropriate variables and control groups and/or control trials. They note that as a consequence '*most blind/placebo controlled trials have yielded negative results..... whereas positive results have been observed where blind/placebo controls have not been employed*'(p243). In a similar vein, Vogler et al (1999) found that only 16 out of a total of 57 retrieved human studies were controlled adequately enough to meet the inclusion criteria of their review of efficacy. A further methodological consideration, as with *Ginkgo biloba,* is the nature of the cognitive instruments used. In the case of demonstrations of cognitive effects, enhancement has been generally restricted to one or two measures from within a disparate battery of tests. No study to date has utilised a computerised assessment battery.

It should also be noted that, by convention, and despite the absence of either a practical or theoretical rationale for doing so, the investigation of the effects of ginseng in humans has been almost exclusively restricted to chronic regimens, with testing typically occurring only after several weeks or months of administration. To the best of our knowledge the present study represents the first investigation into the possibility that single doses of ginseng may modulate cognitive performance.

In light of the foregoing, the current study utilised both a rigorously standardised *Panax ginseng* extract (G115, Pharmaton SA, Switzerland), and an integrated computerised cognitive

assessment battery (CDR). The dose and time dependent mood and cognitive effects of single doses of *Panax ginseng* were investigated in a cohort of 20 healthy young volunteers.

## 3.2. Materials and Methods

### Participants

Fourteen female and six male undergraduate volunteers (mean age 21.3 years, SD 2.64 years) took part in the study, which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation, each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. Of the 20 participants six were light social smokers and they agreed to abstain from smoking on the day of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Cognitive Measures

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive factors were as described in detail previously in Chapter 2 (section 2.2. pages 87-93).

### Subjective mood measure

The 16 Bond-Lader Visual Analogue Scales (Bond and Lader 1974). were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

## Serial Subtraction tasks

Task details and results for the serial subtraction tasks are presented in Chapter 5.

## Treatments

On each study day, participants received six capsules of identical appearance, each containing either an inert placebo or 100 mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland) standardised to a content of 4% ginsenosides. Depending on the condition to which they were allocated on that particular day, the combination corresponded to a dose of either 0 (placebo), 200 mg, 400 mg or 600 mg of *Panax ginseng* extract.

## Procedure

The procedure was identical to that described in Chapter 2 (section 2.2. page 94). Each participant was required to attend a total of five study days that were conducted seven days apart to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment on visits 2 to 5. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader Visual Analogue Scales, followed by the CDR test battery. Following completion of these tasks at each session the serial subtraction tasks (Chapter 5) were completed.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, and the individual task outcome measures making up the factors, were analysed as 'change from baseline' using the SAS statistical package. The initial analysis was made using the general linear models procedure (PROC GLM). Following the recommendations of Keppel (1991) the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three ginseng conditions (200 mg, 400 mg, 600 mg)) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

The three mood outcomes derived from the Bond-Lader scales were analysed using within subjects Analyses of Variance (Minitab) with planned comparisons as per the above.

## 3.3. Results

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 200 mg, 400 mg and 600mg *Panax ginseng* G115) for the cognitive factor scores, and Bond-Lader mood scale scores were subjected to a one-way, repeated measures, analysis of variance. There were no significant differences in baseline performance on any of these measures.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are represented in Table 3.1 Significant results on individual task outcomes are described below in relation to the overall factor to which they contribute (memory task results are presented with either the 'Secondary Memory' or 'Working Memory' factors).

### Cognitive factor outcome measures

Mean baseline raw scores and change from baseline scores on each cognitive factor are presented with graphic representation of the change from baseline data in Figure.3.1.

#### *Quality of Memory measure*

Planned comparisons revealed significant improvements in the accuracy of memory task performance, in comparison to placebo, for 400 mg of ginseng at 1 hour [$t(171) = 2.89$; $p = 0.043$], 2.5 hours [$t(171)=2.25$; $p = 0.026$], 4 hours [$t(171) = 2.12$; $p = 0.035$] and 6 hours post-dose [$t(171) = 3.1$; $p = 0.002$]. A single time point proved significant for 600 mg with performance enhanced at 2.5 hours post-dose [$t(171) = 2.34$; $p = 0.02$]. There were no significant improvements associated with the 200 mg dose of ginseng.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Immediate Word Recall (% accuracy) | placebo | 62.33 3.08 | -10.67 2.18 | -10.00 3.94 | -14.00 3.57 | -11.00 4.70 |
| | 200mg | 61.67 3.34 | -5.83 3.29 | -4.33 3.72 | -2.33 2.62*** | -9.17 3.85 |
| | 400mg | 52.83 3.18 | 0.33 4.37*** | -2.17 3.33* | -2.00 3.99*** | 1.67 3.24**** |
| | 600mg | 54.00 4.56 | -2.50 3.80* | -0.17 3.31** | 0.50 4.35***** | -5.83 4.48 |
| Simple Reaction time (msecs) | placebo | 249.26 8.53 | 5.72 4.57 | 4.34 4.36 | -1.42 4.13 | 0.17 5.14 |
| | 200mg | 242.09 5.59 | 10.27 5.17 | 7.54 6.63 | 9.46 5.400 | 7.93 6.04 |
| | 400 mg | 242.27 6.46 | 12.77 5.29 | 8.30 3.99 | 6.25 5.50 | 13.39 4.92? |
| | 600mg | 245.95 5.13 | 6.54 6.84 | 3.03 5.24 | 9.53 5.50? | 16.96 6.61? |
| Digit Vigilance Accuracy (%) | placebo | 98.00 1.09 | -1.33 1.85 | 1.00 1.11 | -0.67 1.17 | -0.67 0.82 |
| | 200mg | 98.33 0.82 | -0.67 1.36 | -2.00 1.61* | -0.67 1.27 | -0.33 1.02 |
| | 400mg | 96.67 1.03 | 2.00 1.38* | 2.00 1.19 | 2.00 0.98* | 0.67 1.52 |
| | 600mg | 99.00 0.73 | -0.67 1.17 | -1.67 1.36* | -1.33 0.92 | -1.67 1.44 |
| Digit Vigilance False alarms (number) | placebo | 0.15 0.08 | 0.10 0.14 | 0.10 0.16 | 0.35 0.15 | 0.25 0.18 |
| | 200mg | 0.55 0.15 | -0.05 0.30 | 0.05 0.18 | 0.25 0.26 | -0.40 0.15** |
| | 400mg | 0.50 0.20 | -0.15 0.22 | -0.10 0.23 | -0.20 0.24** | -0.10 0.20 |
| | 600mg | 0.50 0.17 | 0.00 0.16 | 0.15 0.18 | -0.15 0.23* | 0.00 0.32 |
| Digit Vigilance Reaction time (msecs) | placebo | 406.27 11.13 | -3.54 8.42 | -3.51 6.56 | -9.44 6.43 | -2.14 8.60 |
| | 200mg | 388.67 8.79 | 5.00 7.45 | 4.88 9.03 | 20.08 9.09*** | 15.19 6.18* |
| | 400mg | 408.02 6.87 | -5.59 6.62 | -6.78 6.05 | -3.39 5.62 | -1.60 7.28 |
| | 600mg | 387.38 9.85 | 8.04 8.99 | 11.27 9.26 | 13.43 9.58** | 26.30 9.11*** |
| Choice reaction time accuracy (%) | placebo | 94.50 1.17 | 0.50 1.15 | -0.40 1.17 | -2.10 1.49 | -1.70 1.68 |
| | 200mg | 93.40 0.97 | 0.70 0.86 | 0.70 0.92 | -0.50 0.74 | 0.10 0.83 |
| | 400mg | 96.10 0.75 | -1.30 0.99 | -1.70 1.47 | -2.30 0.93 | -1.80 1.01 |
| | 600mg | 94.50 1.33 | -0.30 0.94 | -0.80 0.89 | -1.20 0.84 | -2.70 1.15 |
| Choice reactionTime (msecs) | placebo | 401.14 10.70 | -2.95 5.84 | -5.32 8.34 | -14.30 10.00 | -16.16 8.34 |
| | 200mg | 391.31 8.53 | 11.43 5.36* | -0.82 6.57 | 5.49 8.04** | 8.57 8.88*** |
| | 400mg | 400.80 12.60 | -0.19 6.41 | -2.47 5.69 | -4.89 7.62 | -14.43 6.23 |
| | 600mg | 398.80 8.52 | -8.36 6.05 | -8.83 5.98 | -3.10 8.43 | -8.99 6.14 |
| Spatial Memory (%>chance) | placebo | 92.31 1.32 | -1.88 1.89 | 1.00 1.55 | -2.38 2.19 | -8.63 6.61 |
| | 200mg | 88.31 3.36 | 1.75 3.92 | 0.38 3.67 | -0.88 4.15 | -13.13 10.03 |
| | 400mg | 91.63 1.87 | -1.69 2.37 | -0.75 1.86 | -2.81 3.08 | -2.44 2.36 |
| | 600mg | 92.44 1.30 | -5.25 5.33 | -1.56 1.69 | -8.25 4.36 | -7.69 3.59 |
| Spatial memory Reaction time (msecs) | placebo | 564.92 17.84 | -23.54 18.15 | -42.64 17.20 | -56.31 15.21 | -22.01 17.30 |
| | 200mg | 535.65 18.63 | 14.62 20.29* | 4.10 15.72* | -0.35 14.79** | -4.00 9.51 |
| | 400mg | 558.03 20.79 | -31.54 11.67 | -13.95 30.50 | -37.08 19.82 | -17.12 22.06 |
| | 600mg | 568.54 28.01 | -39.70 21.07 | -49.94 14.74 | -36.47 24.38 | -40.29 22.73 |
| NumericWork'g Memory (%>chance) | placebo | 88.89 1.76 | -4.78 1.90 | -1.78 1.35 | -3.33 1.88 | -2.22 1.26 |
| | 200mg | 89.00 1.81 | -3.67 2.72 | 1.33 1.33 | -3.56 1.99 | -2.44 1.54 |
| | 400mg | 88.67 1.84 | -1.33 1.75 | -3.89 1.94 | -2.78 1.84 | -2.89 1.68 |
| | 600mg | 89.33 1.50 | -4.00 1.55 | -1.11 1.84 | -3.89 1.43 | -4.45 1.66 |
| Numeric Working Memory Reaction Time (msecs) | placebo | 535.39 19.07 | -3.82 9.18 | -16.00 11.71 | -3.30 13.81 | -28.71 13.85 |
| | 200mg | 527.71 19.71 | -16.56 9.42 | -13.52 12.86 | -5.48 11.99 | -30.25 12.41 |
| | 400mg | 533.37 22.17 | -21.32 11.15 | -24.54 9.92 | -7.36 9.10 | -30.66 10.73 |
| | 600mg | 527.50 20.10 | -11.49 9.90 | -30.44 12.22 | -25.03 8.47 | -27.52 9.61 |
| Delayed Word Recall (% accuracy) | placebo | 43.67 4.00 | -12.17 3.21 | -17.33 4.79 | -13.50 3.45 | -20.00 4.52 |
| | 200mg | 47.50 4.45 | -13.33 4.02 | -15.50 4.16 | -13.00 4.58 | -17.33 4.32 |
| | 400mg | 39.00 2.87 | -9.83 5.47 | -8.17 4.63* | -10.50 4.13 | -15.00 5.55 |
| | 600mg | 41.67 4.57 | -12.17 3.81 | -12.50 3.44 | -12.67 4.32 | -13.67 5.78 |
| Word Recognition (%>chance) | placebo | 72.73 2.99 | -8.06 4.00 | -14.06 3.75 | -12.04 4.31 | -15.73 5.11 |
| | 200mg | 66.67 4.30 | -2.67 4.03 | -5.33 2.72 | -4.56 4.59 | -6.86 3.95 |
| | 400mg | 63.75 4.70 | 1.58 4.22* | -0.08 5.90** | -5.71 4.18 | -2.08 5.96** |
| | 600mg | 63.00 4.97 | -4.67 4.25 | -1.33 4.70** | -5.67 5.06 | -6.00 4.46* |
| Word Recognition Reaction time (msecs) | placebo | 666.61 24.65 | -4.61 31.42 | 11.82 34.88 | -4.91 25.55 | -4.97 28.57 |
| | 200mg | 642.56 29.39 | 19.79 14.95 | -0.70 16.59 | 10.92 21.86 | 12.29 17.28 |
| | 400mg | 646.38 21.11 | 10.99 12.33 | 25.59 21.18 | 35.13 27.49 | 24.11 24.50 |
| | 600mg | 669.16 44.33 | 23.68 20.23 | -13.05 23.68 | -32.02 19.01 | -10.64 32.61 |
| Picture Recognition (%>chance) | placebo | 75.25 4.48 | -10.75 4.23 | -8.50 3.96 | -11.00 5.46 | -8.50 4.87 |
| | 200mg | 67.75 3.88 | -0.25 4.19* | -5.25 4.97 | -8.25 5.85 | -2.75 5.30 |
| | 400mg | 69.50 4.34 | -0.25 4.45* | -6.75 6.55 | -5.25 4.03 | -5.50 4.66 |
| | 600mg | 71.75 3.77 | -0.75 4.06 | -4.00 3.75 | -9.75 4.31 | -18.00 5.25 |
| Picture recognit'n Reaction time (msecs) | placebo | 743.16 24.17 | 19.69 19.43 | 10.74 23.44 | -6.83 25.92 | -19.76 21.54 |
| | 200mg | 727.40 22.51 | 4.93 11.65 | -2.75 19.71 | 30.25 24.12 | 18.24 20.37* |
| | 400mg | 755.33 33.81 | 1.76 14.30 | 1.15 21.89 | -15.10 24.54 | -14.07 23.31 |
| | 600mg | 739.48 29.57 | 9.21 16.72 | -7.02 19.81 | -3.19 21.10 | -37.90 21.73 |

*Table 3.1. Effects of Panax ginseng (G115) on individual task outcome measures from the CDR battery.. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, ****,= 0.001,*****, = 0.0005 compared to placebo).*

### *Secondary Memory Factor*

On this factor performance was enhanced by 600 mg of ginseng at 1 hour [$t(171) = 2.01$; $p = 0.046$], 2.5 hours [$t(171) = 2.97$; $p = 0.0034$],and 4 hours post dose [$t(171) = 2.13$; $p = 0.034$]. Improvements were also seen at all time points for 400 mg of ginseng (1 hour [$t(171) = 3.11$; $p = 0.0022$], 2.5 hours [$t(171) = 3.04$; $p = 0.0027$], 4 hours [$t(171) = 2.52$; $p = 0.013$] and 6 hours post-dose [$t(171) = 3.19$; $p = 0.0036$]), but were restricted to 4 hours post dose for 200 mg of ginseng [$t(171) = 2.13$; $p = 0.039$].

Analysis of the component tasks revealed that all four single task measures loading on the 'Secondary Memory' factor evinced significant improvements in comparison to placebo following ginseng administration. This effect was most pronounced for the immediate word recall task, with improvements at all time points following 400mg (1 hour [$t(171) = 2.07$; $p = 0.0034$], 2.5 hours [$t(171) = 2.12$; $p = 0.036$], 4 hours [$t(171) = 3.24$; $p = 0.0014$] and 6 hours [$t(171) = 3.42$; $p = 0.0008$)],and following 600mg at 1 hour [$t(171) = 2.2$; $p = 0.028$], 2.5 hours [$t(171) = 2.66$; $p = 0.009$] and 4 hours post-dose [$t(171) = 3.92$; $p = 0.0001$]. There was also a single significant improvement following 200mg at 4 hours post-dose [$t(171 = 3.15$, $p = 0.002$]. A consistent pattern also emerged on the delayed word recognition task with significant improvements evident following 400mg at 1 hour [$t(171) = 2.12$; $p = 0.036$], 2.5 hours [$t(171) = 3.07$; $p = 0.003$] and 6 hours post-dose [$t(171) = 2.99$, $p = 0.003$], and following 600mg at 2.5 hours [$t(171) = 2.79$; $p = 0.006$] and 6 hours post-dose [$t(171) = 2.13$; $p = 0.034$] The delayed picture recognition task was improved at 1 hour for both 200 and 400 mg [$t(171) = 2.06$; $p = 0.04$, in both cases], and delayed word recall was significantly improved for the 400mg dose at a single time point (2.5 hours [$t(171) = 2.13$; $p = 0.034$]).

***Working Memory Factor***

There were no significant differences in comparison to placebo on this factor for any of the doses of ginseng at any of the post-dose time points. There were no significant improvements on the component measures of the 'Working Memory' factor.

***Speed of Memory factor***

Significant differences in the speed of performance on the memory tasks were restricted to a decrement in speed for 200 mg of ginseng at 4 hours post-dose [$t(171) = 2.87$; $p = 0.0045$].

In keeping with this, the task outcomes making up the 'Speed of Memory' factor were unaffected for the 400 and 600 mg doses. However, following the 200 mg dose speed of performance was impaired during the delayed picture recognition task at 6 hours post-dose [$t(171) = 2.01$; $p = 0.046$] and during the spatial memory task at 1 hour [$t(171) = 1.99$; $p = 0.047$], 2.5 hours [$t(171) = 2.45$; $p = 0.015$] and 4 hours post-dose [$t(171)=2.93$; $p = 0.0039$].

***Speed of Attention factor***

There were significant decrements in the speed of performance of the attentional tasks, in comparison to placebo, following both 200 mg and 600 mg of ginseng at both 4 hours ([$t(171) = 4.21$; $p = 0.0001$] and [$t(171) = 3.15$; $p = 0.0019$] respectively), and 6 hours post dose ( [$t(171) = 3.48$; $p = 0.0006$] and [$t(171) = 3.66$; $p = 0.0003$] respectively). Speed was not, however, significantly affected for the 400 mg dose of ginseng.

In keeping with the factor results all three tasks making up the 'Speed of Attention' factor showed decrements. Speed of performance on the simple reaction time task was significantly reduced following 600 mg at 4 hours [$t(171) = 1.97$; $p = 0.05$] and following both 400mg and 600mg at 6 hours post-dose ([$t(171) = 2.38$; $p = 0.018$]and [$t(171) = 3.03$; $p = 0.0028$]. The 200mg dose evinced decrements on the choice reaction time task at 1 hour [$t(171) = 2.13$; $p = 0.035$], 4 hours [$t(171) = 2.93$; $p = 0.004$] and 6 hours post-dose [$t(171) = 3.66$; $p = 0.0003$],

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Quality of Memory (%X6) | placebo | 435.18 9.93 | -48.30 9.41 | -50.67 11.94 | -56.25 11.57 | -66.08 14.93 |
| | 200mg | 420.90 13.47 | -24.00 10.65 | -28.71 11.28 | -32.58 13.29 | -51.68 11.13 |
| | 400mg | 405.38 12.27 | -11.19 13.16 | -21.81 14.32 | -29.05 11.19 | -26.24 13.06 |
| | 600mg | 412.19 13.15 | -29.33 10.25 | -20.67 10.30 | -39.72 14.14 | -55.63 15.60 |
| Secondary Memory (%X4) | placebo | 253.98 8.74 | -41.65 8.80 | -49.90 10.63 | -50.54 10.67 | -55.23 11.23 |
| | 200mg | 243.58 11.73 | -22.08 8.04 | -30.42 10.43 | -28.15 10.84 | -36.11 9.56 |
| | 400mg | 225.08 10.74 | -8.17 12.26 | -17.17 13.41 | -23.46 11.01 | -20.92 12.62 |
| | 600mg | 230.41 12.50 | -20.08 9.87 | -18.00 10.28 | -27.58 12.05 | -43.50 13.26 |
| Working Memory (%X2) | placebo | 181.20 2.80 | -6.65 2.99 | -0.78 2.24 | -5.71 3.54 | -10.85 6.97 |
| | 200mg | 177.31 4.16 | -1.92 5.57 | 1.71 4.21 | -4.43 5.38 | -15.57 10.31 |
| | 400mg | 180.29 3.24 | -3.02 2.81 | -4.64 2.54 | -5.59 3.24 | -5.33 3.21 |
| | 600mg | 181.77 2.41 | -9.25 5.20 | -2.67 2.59 | -12.14 4.81 | -12.13 4.53 |
| Speed of Memory (summed msecs) | placebo | 2509.1 68.41 | -12.27 50.15 | -35.08 58.92 | -71.35 52.52 | -75.45 51.13 |
| | 200mg | 2433.3 74.46 | 22.79 37.92 | -12.88 45.22 | 35.34 45.05 | -3.72 41.23 |
| | 400mg | 2493.1 84.07 | -40.11 28.20 | -11.74 43.41 | -24.42 33.94 | -37.74 33.51 |
| | 600mg | 2504.7 102.65.. | -18.30 42.79 | -100.5 47.79 | -96.72 43.16 | -116.4 50.23 |
| Quality of Attention (%) | placebo | 91.20 0.75 | -0.45 0.91 | 0.15 0.77 | -1.70 0.91 | -1.40 0.99 |
| | 200mg | 90.40 0.60 | 0.10 0.87 | -0.60 1.02 | -0.80 0.82 | 0.30 0.61 |
| | 400mg | 90.55 0.67 | 0.40 0.83 | 0.15 1.06 | -0.05 0.65 | -0.50 0.82 |
| | 600mg | 91.30 0.77 | -0.45 0.61 | -1.30 0.64 | -1.05 0.60 | -2.10 1.10 |
| Speed of Attention (summed msecs) | placebo | 1055.7 26.37 | -0.77 13.44 | -4.49 13.75 | -25.16 14.37 | -18.13 15.32 |
| | 200mg | 1022.1 19.25 | 26.69 12.53 | 11.61 14.80 | 35.01 18.98 | 31.68 13.53 |
| | 400mg | 1051.1 22.09 | 6.99 12.19 | -0.94 10.56 | -2.03 10.66 | -2.64 11.89 |
| | 600mg | 1032.1 18.09 | 6.23 17.28 | 5.46 15.47 | 19.86 17.18 | 34.28 15.68 |

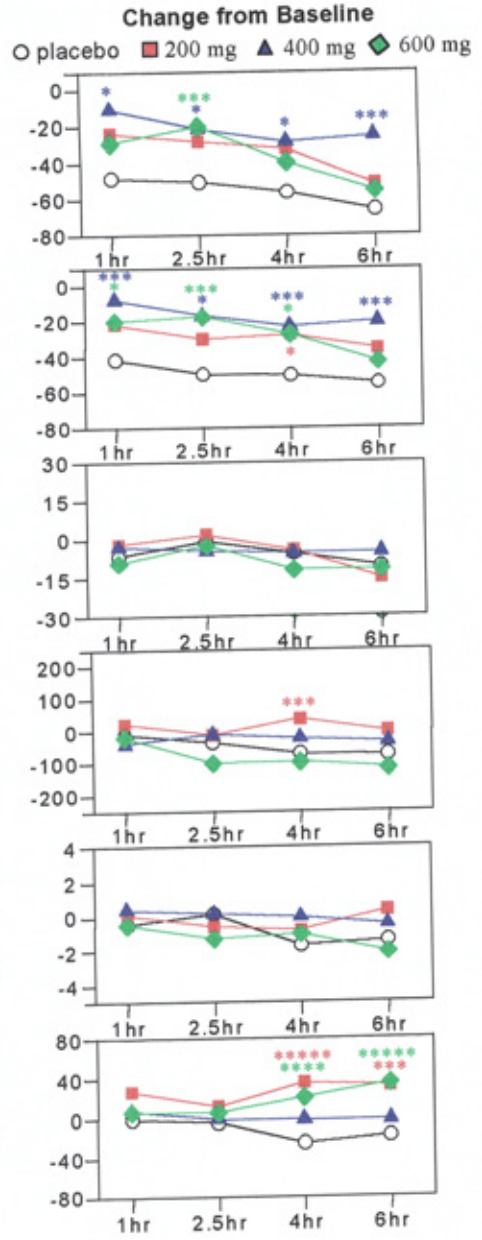


*Figure 3.1 Effects of Panax ginseng (G115) on cognitive measures;'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Accuracy of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of ginseng. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01,***, p = 0.005; ****, p = 0.001; *****, p = 0.0005 compared to the corresponding placebo score).Units are as per the table.*

and both 200mg and 600mg resulted in slower performance on the digit viglance task at 4 and 6 hours post-dose (200mg- 4 hours [t(171) = 3.76; p = 0.0002] and 6 hours [t(171) = 2.2; p = 0.028]. 600mg - 4 hours [t(171) = 2.92; p = 0.004] and 6 hours [t(171) = 3.63; p = 0.0004]).

*Accuracy of attention factor*

There was a single significant enhancement in accuracy of performance on the attention tasks, which was restricted to the 200 mg dose at 6 hours post-dose [$t$(171) = 1.99; p = 0.048]. Given the nature of the statistical analysis it seems prudent not to over-interpret this.

Results were somewhat mixed on the single tasks making up this factor, with decrements evident on accuracy of performance of the digit vigilance task for both 200mg and 600mg at 2.5 hours([t(171) = 2.3; p = 0.023] and [t(171) = 2.04; p = 0.043] respectively). In contrast the only dose not to result in speed deficits on the same task (400mg) resulted in improved accuracy of performance at 1 and 4 hours post-dose ([t(171) = 3.55; p = 0.012] and [t(171) = 2.04; p = 0.043] respectively). However, all three doses were associated with improved performance in terms of reduced false alarms on the same task at one time point, 6 hours in the case of the 200mg dose [t(171) = 2.29; p = 0.023], and 4 hours for both 400mg [t(171) = 2.61; p = 0.01] and 600mg [t(171) = 2.37; p = 0.019] of ginseng.

**Subjective mood measures**

Both the 200 mg and 400 mg doses of ginseng were associated with a significant reduction in scores on the 'alert' factor derived from the Bond-Lader visual analogue scales during the 6 hours post-dose testing session ([t(171) = 3.66, p = 0.001] and [t(171) = 2.94, p = 0.01] respectively). Whilst there was some evidence of 'calmness' improving in comparison to placebo at the same time point for the same doses, this effect did not reach significance.

There were also no significant differences on the 'content' factors.

The effects of ginseng on mood measures are presented in the table and graphs of Figure 3.2.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| 'Alert' | placebo | 52.24 4.02 | 5.3 2.45 | 4.95 3.12 | 8.38 3.64 | 7.18 3.77 |
| | 200mg | 59.46 3.64 | 6.32 2.5 | 3.21 3.97 | 3.98 3.68 | -2.07 4.24 |
| | 400mg | 62.98 3.02 | 3.32 2.72 | 5.92 2.71 | 3.62 3.71 | -0.25 4.31 |
| | 600mg | 51.93 3.95 | 5.07 3.98 | 9.46 3.62 | 7.27 3.92 | 7.76 4.45 |
| 'Calm' | placebo | 63.68 3.25 | -1.1 2.18 | -3.95 2.35 | -3.3 2.31 | -7.03 2.53 |
| | 200mg | 62.85 3.93 | 1.68 3.19 | 1.83 4.73 | 2.28 3.43 | 1.03 4.43 |
| | 400mg | 62.43 3.62 | -0.2 3.13 | -2.4 3.89 | -1.88 3.17 | 0.65 3.83 |
| | 600mg | 64.33 3.17 | -3.25 2.54 | -2.4 3.29 | -4.83 2.84 | -8.6 3.81 |
| 'Content' | placebo | 63.62 3.65 | 1.41 3.45 | 0.97 2.35 | 2.28 2.23 | 3.83 2.64 |
| | 200mg | 64.82 3.99 | 5.75 2.55 | 2.6 3.75 | 5.85 3.15 | 2.65 3.71 |
| | 400mg | 67.87 3.29 | 0.29 2.83 | 0.01 2.37 | 1.13 2.37 | 0.73 3.57 |
| | 600mg | 61.29 3.9 | -1.78 3.97 | 3.74 2.16 | 3.2 3.35 | 3.46 4.04 |

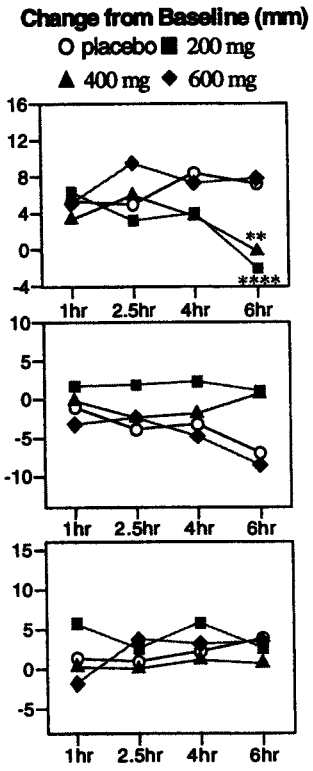


*Figure 3.2 Effects of ginseng on self-rated mood as measured using Bond-Lader visual analogue scales. The table presents raw scores and change from baseline scores for each dose of ginseng (means with standard errors in italics). Graphs represent the change from baseline scores for the three mood dimensions: 'alert', 'calm' and 'content' (**, $p < 0.01$; ****, $p < 0.001$ compared to the corresponding placebo score).*

### 3.4. Discussion

The results of the current study show that ingestion of *Panax ginseng* can affect cognitive performance in a time and dose-dependent manner. Moreover, these modulatory effects were evinced by single doses of ginseng. As far as we are aware this is the first investigation to demonstrate such an effect.

The most striking finding was the ginseng-associated improvement in memory. The 'Quality of Memory' measure, comprised of accuracy scores for all of the memory tasks in the battery, was enhanced at all time points following 400 mg of ginseng (a similar effect was observed following the 600 mg dose but at 1 hour only). Further breakdown of the 'Quality of Memory' measure revealed that this enhancement was restricted to the 'Secondary Memory' factor (which comprises measures of immediate and delayed word recall, delayed picture and word recognition). No improvement was seen on the 'Working Memory' factor (which comprises accuracy scores from the spatial and numeric working memory tasks). This enhancement of 'Secondary Memory' was again evident following 400 mg ginseng at all time points, and also following 600 mg at all but the 6 hour session. There was also a single improvement for 200 mg at 4 hours post-dose. This would seem to suggest that the mnemonic effect of ginseng preferentially targets some or all aspects of learning, consolidation and/or retrieval of information, rather than aspects of working memory. Clearly this apparent fractionation of memory sub-systems by ginseng merits further investigation.

In contrast to enhanced performance on memory tasks, time and dose dependent decrements were observed in performance on the 'Speed of Attention' factor, comprising reaction times from three tasks assessing attention. These, however, were restricted to the later testing sessions i.e. those at 4 and 6 hours post-dose, and were apparent only for the 200 mg and 600 mg doses of ginseng. The decrement for the 200 mg dose was accompanied by a subjective reduction in self-reported 'alertness' on the Bond Lader visual analogue scales, which reached significance

by the 6 hour testing session. The dose exhibiting the most marked and consistent mnemonic effects, 400 mg, was not associated with any decrement on the 'Speed of Attention' factor, but was associated with a significant reduction in 'alertness', again at the 6 hour testing session.

Examination of the individual task outcomes (Table 3.1) reveals a pattern of effects which is remarkably consistent with that observed for the factors. For example there were improvements in all measures contributing to the 'Secondary Memory' factor following the 400 mg dose, at one or more time points, with less pronounced improvements for the 600 mg dose. Inspection of the tasks contributing to the 'Speed of Attention' factor revealed a similar pattern (Table 1). Thus both 200 mg and 600 mg ginseng resulted in a slowing of responses in the digit vigilance task at both 4 and 6 hours. At the same time points simple and choice reaction time were differentially slowed by the 600 mg and 200 mg dose respectively.

The overall pattern of both cognitive costs and benefits is somewhat in keeping with the overall tenor of the literature pertaining to ginseng. For instance ginseng extracts have been shown to have opposing effects on a number of physiological parameters. Examples include: time-dependent, opposite effects on blood pressure (Wood *et al,* 1964); opposing effects on vasoconstriction depending on dose and target vessel (Lee *et al,* 1981, Lei and Chiou, 1986); and opposite species-specific modulatory effects on the Hypothalamic-Pituitary-Adrenal response to stress (Luo *et al,* 1993). Similarly Bahrke and Morgan (1994) note both hyper-tensive and hypo-tensive effects, histamine and antihistamine-like actions and stimulant and depressant CNS activity in reports from relevant animal research. Some of this inconsistency within the literature can certainly be attributed to the wide variety of extracts, components, and doses used. Moreover even rigorous research in this area using standardised extracts rarely generates simple dose-dependent relationships. The current study found improvements in memory which were most marked for the middle dose under investigation, with decrements in reaction times on attention tasks which were restricted to the lowest and highest doses utilised. Such a pattern of results supports the contention that the equivocal and less than compelling evidence for the efficacy of ginseng in humans (Bahrke and Morgan, 2000; Vogler *et al,* 1999)

may in part be related to the wide range of doses and extracts utilised. Such findings reinforce the need for carefully controlled, multiple-dose, repeated testing regimes particularly in the early stages of investigating the cognitive effects of such agents.

This intriguing pattern of results necessarily raises the question of possible mechanisms. The possibility that the effects demonstrated here represent a modulation of cholinergic function receives some support from observations of ginseng related amelioration of memory decrements following scopolamine administration to rodents (Sloley *et al,* 1999), and demonstrations of *in vitro* nicotinic and muscarinic receptor binding activity (Lewis *et al,* 1999). However, simple antagonism and agonism of the cholinergic system does not produce a pattern of results consistent with that seen here. For example scopolamine produced a global impairment of all of the tasks from the CDR battery that Wesnes *et al's* (1998) study had in common with the present study, and similar deficits are typically reduced by nicotine, which can itself enhance both attentional and mnemonic performance (e.g. Rusted and Warburton, 1989; Wesnes *et al,* 1988). It does seem unlikely that the same mechanism could be responsible for both the memory enhancement and the decrements in performance of the 'Speed of Attention' factor evinced here. Taken with the (unexpected) reductions in alertness at the 6 hour testing session it seems plausible that a number of different, as yet unidentified, mechanisms are at work.

This leads inevitably to the question of which of ginseng's components underlie its neuroactive properties. Certainly there are numerous constituents of ginseng, which, alone or in combination, may contribute to its efficacy. In particular, the functional nature of the ginsenosides is of obvious academic interest. However, it also seems reasonable to suggest that the focus of research in this area may best be directed towards the effects of standardised whole extracts of ginseng, especially given their current widespread use.

It is also interesting to note that the effects of chronic administration of a *Panax ginseng/Ginkgo biloba* combination to both healthy sufferers from neurasthenic complaints (Wesnes *et al,* 1997), and healthy middle aged volunteers (Wesnes *et al,* 2000) have been

investigated using the CDR battery and cognitive domain factors utilised here. In both studies significant differences were restricted to treatment-associated improvements in memory performance (Quality of Memory' measure), with no decrements on the other cognitive domain factors, or on subjective mood measures. In light of the demonstration of significant improvements on the 'Speed of Attention' factor, with less marked improvements in mnemonic performance, following single doses of *Ginkgo biloba* (Chapter 2), it may be of interest to investigate the effects of single doses of a ginseng/ginkgo combination in young volunteers in order to establish whether the combination produces mutually-antagonising, additive or synergistic effects.

In conclusion, the results of the current study show for the first time that single doses of ginseng are capable of a dose dependent modulation of cognitive performance in healthy young adults. Whilst this modulation was, on balance, overwhelmingly beneficial for the middle dose studied (400 mg), there was evidence of cognitive and subjective mood costs associated with other doses under investigation.

# CHAPTER 4. THE COGNITIVE AND MOOD EFFECTS OF ACUTE ADMINISTRATION OF A *GINKGO BILOBA/PANAX GINSENG* COMBINATION

## 4.1. Introduction

A small number of studies have examined the effects of a combination of *Ginkgo biloba* and *Panax ginseng*. In each case they used a 60:100 combination of ginkgo GK501 and ginseng G115. Findings include improvements in retention of learned behaviour on a number of tasks for both young and old rats following ingestion of the combination (Petkov *et al,* 1993), and a similar inhibition of platelet aggregation and increase in erythrocyte velocity in young volunteers (Kieswetter *et al,* 1992) as had previously been demonstrated (by the same research team) following ingestion of *Ginkgo biloba* (Jung *et al,* 1990).

Two double-blind, placebo controlled clinical trials also assessed the cognitive effects of chronic regimens of the *Ginkgo biloba/Panax ginseng* combination in humans, using the same tailored version of the CDR battery and factor structure as utilised in Chapters 2 and 3 of the current thesis. Both of these studies demonstrated improvements on the global 'Quality of Memory' measure. In the case of the first study (Wesnes *et al,* 1997), 64 patients suffering from neurasthenia, an age related condition with a possible cerebro-vascular aetiology (analogous to 'cerebral insufficiency'), received twice-daily doses totalling 80 mg, 160 mg, or 320 mg of the combination or a placebo. All three doses were associated with memory improvements on at least two of the 1 hour post morning dose testing sessions on the 1$^{st}$ day, 30$^{th}$ day and 90$^{th}$ day following commencement of treatment. Curiously, occasional impairment on the same measure was evident following the higher doses at the 1 hour post afternoon dose testing sessions.

In order to extend this line of research, and clarify the possibility of a bi-phasic effect, a further, larger, double-blind, multi-centre, clinical trial was undertaken (Wesnes *et al,* 2000). This study utilised a cohort of 256 healthy middle-aged participants randomly allocated to 3 conditions: 160mg of the combination twice daily; 320 mg of the combination once daily, and placebo.

Testing took place four times daily (1 hour pre-dose and 1, 3, and 6 hours post-dose) prior to commencement of the treatment and at 4, 8, 12 and 14 weeks after commencement of treatment. Improvements on the 'Quality of Memory' measure were evident at 1 hour and 6 hours (the latter corresponds to the testing session which evinced occasional decrements in the first study) following the daily or first dose of the combination. No improvement was evident at 3 hours post-dose or at the pre-dose testing session. This lack of an improvement prior to the days treatment would seem to indicate that the enhancement may well have been as a result of the acute effects of the days treatments, rather than an accumulative effect of the ginkgo/ginseng combination over time. This latter possibility is also strengthened by the observation of a significant improvement on the 'Quality of Memory' measure 1 hour after the first dose on the first day of the original study (Wesnes *et al,* 1997).

In light both of the above outlined research, and the differing patterns of results in Chapters 2 and 3, which saw ginkgo being most strongly associated with increased speed on attentional tasks, whilst different doses of ginseng were most strongly associated either with memory improvement, or reduced speed on attentional tasks, it seems apposite to investigate the cognitive effects of the product combining the two extracts. To this end the current study, methodologically identical to the previous two studies, represents an investigation of the possibility of opposing, additive, or synergistic cognitive effects following administration of single doses of 320 mg, 640 mg and 960 mg of the *Ginkgo/Ginseng* combination to healthy young volunteers.

## 4.2.Materials and Methods

### Participants

20 undergraduate volunteers (10 male, 10 female, mean age 20.6 years, SD 4.2) took part in the study, which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the study. Of the 20 participants two were light social smokers and they agreed to abstain from smoking on the day of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Cognitive Measures

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive factors were as described in detail previously in Chapter 2 (section 2.2. pages 87-93).

### Subjective mood measure

The 16 Bond-Lader Visual Analogue Scales (Bond and Lader 1974) were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

### Serial Subtraction tasks

Task details and results for the serial subtraction tasks are presented in Chapter 5.

**Treatments**

On each study day participants received six capsules of identical appearance, each containing either an inert placebo or a combination of 60mg *Ginkgo biloba* extract (GK501, Pharmaton SA, Switzerland) and 100mg of *Panax ginseng* extract (G115, Pharmaton SA, Switzerland). Depending on the condition to which they were allocated on that particular day the combination of capsules corresponded to a dose of either 0 mg (placebo), 320 mg, 640 mg, or 960 mg of the *Ginkgo biloba/Panax ginseng* combination.

**Procedure**

The procedure was identical to that described in Chapter 2 (section 2.2. page 94). Each participant was required to attend a total of five study days that were conducted seven days apart to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session that established baseline performance for that day, and was immediately followed by the day's treatment on days 2 to 5. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader Visual Analogue Scales, followed by the CDR test battery. Following completion of these tasks at each session the serial subtraction tasks (Chapter 5) were completed.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, and the individual task outcome measures making up the factors, were analysed as 'change from baseline' using the SAS statistical package. The initial analysis was made using the general linear models procedure (PROC GLM). Following the recommendations of Keppel (1991) the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three ginkgo/ginseng conditions (320 mg, 640 mg and 960 mg) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

The three mood outcomes derived from the Bond-Lader scales were analysed using within subjects Analyses of Variance (Minitab) with planned comparisons as per the above.

## 4.3. Results

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 320 mg, 640 mg and 960 mg of the ginkgo/ginseng combination) for the cognitive factor scores, and Bond-Lader mood scale scores were subjected to a one-way, repeated measures ANOVA. There were no significant differences in baseline performance on any of these measures.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are represented in Table 4.1. Significant results on individual task outcomes are described below in relation to the overall factor to which they contribute (memory task results are presented with either the 'Secondary Memory' or 'Working Memory' factors to which they contribute).

### Cognitive factor outcome measures

Mean baseline raw scores and change from baseline scores on the 'Quality of Memory' measure and five cognitive factors are presented, with graphic representation of the change from baseline data, in Figure.4.1.

#### *Quality of Memory measure*

Planned comparisons revealed significant improvements in the accuracy of memory task performance, in comparison to placebo, for 960 mg of the combination at 1 hour [$t(171) = 3.39$; $p = 0.0009$], and 6 hours [$t(171) = 3.13$; $p = 0.002$] post-dose. There was also a trend towards improved performance on the combined components of this factor for 640 mg of the combination at 6 hours post dose [$t(171) = 1.86$; $p = 0.065$]. There were no significant improvements associated with the 320mg dose.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Immediate Word Recall (% accuracy) | Placebo | 64.00 3.39 | -9.33 3.38 | -9.33 4.77 | -9.67 3.35 | -12.33 3.68 |
| | 320mg | 54.83 2.56 | 0.50 2.46* | -3.50 2.58 | 2.00 4.23*** | -0.50 2.03*** |
| | 640mg | 56.00 3.99 | -2.33 3.88 | -0.50 3.53* | -1.17 3.70* | -3.33 3.78* |
| | 960mg | 56.17 4.47 | 0.17 4.60* | -7.50 5.56 | 0.33 3.91* | -0.17 4.72*** |
| Simple Reaction time (msecs) | Placebo | 257.72 10.28 | 8.96 6.92 | 7.59 5.46 | 1.81 5.43 | 13.48 6.02 |
| | 320mg | 247.80 6.61 | 12.83 6.38 | 8.59 6.74 | 8.82 7.30 | 21.35 8.97 |
| | 640mg | 255.37 9.14 | 11.48 5.96 | 7.46 7.70 | 16.10 6.73* | 15.35 11.07 |
| | 960mg | 253.15 9.59 | 4.60 6.16 | -1.47 5.80 | 8.87 8.73 | 6.93 8.54 |
| Digit Vigilance Accuracy (%) | Placebo | 98.00 0.98 | -0.33 1.23 | 0.00 1.18 | -0.67 1.36 | -1.33 1.78 |
| | 320mg | 97.00 1.02 | 1.67 1.07 | 1.67 0.95 | 1.33 1.33 | 0.67 1.44 |
| | 640mg | 98.67 0.61 | -0.33 1.13 | -1.67 1.17 | -1.33 1.33 | -2.00 1.61 |
| | 960mg | 98.00 0.85 | -0.33 1.13 | -0.33 0.90 | -0.33 1.32 | -1.33 1.04 |
| Digit Vigilance False alarms (number) | Placebo | 0.55 0.17 | -0.45 0.17 | 0.00 0.21 | -0.25 0.23 | -0.30 0.21 |
| | 320mg | 0.45 0.14 | 0.00 0.21 | -0.10 0.19 | -0.05 0.15 | 0.05 0.21 |
| | 640mg | 0.30 0.13 | 0.30 0.23 | 0.30 0.21 | 0.45 0.28 | 0.25 0.16 |
| | 960mg | 0.55 0.17 | -0.15 0.18 | 0.10 0.19 | 0.10 0.24 | 0.00 0.23 |
| Digit Vigilance Reaction time (msecs) | Placebo | 384.66 12.62 | 4.16 5.32 | -1.16 6.44 | 1.38 8.49 | 1.18 8.28 |
| | 320mg | 362.44 9.22 | 25.89 8.94*** | 24.34 6.68*** | 26.90 7.54*** | 42.21 9.5***** |
| | 640mg | 380.28 10.34 | 0.03 5.77 | 8.05 5.48 | 22.50 6.70** | 9.83 5.74 |
| | 960mg | 378.05 10.63 | 5.58 7.23 | -0.92 5.16 | 12.63 6.96 | 1.72 6.22 |
| Choice reaction time accuracy (%) | Placebo | 94.40 0.69 | 0.10 0.83 | -0.10 0.95 | -1.10 1.00 | -1.40 0.76 |
| | 320mg | 95.40 0.81 | -0.80 0.96 | -2.20 0.71* | -1.70 0.86 | -3.80 1.20** |
| | 640mg | 93.00 1.24 | 1.50 1.05 | -0.50 0.97 | -1.00 0.93 | -1.70 1.21 |
| | 960mg | 95.20 0.57 | -0.30 0.86 | -2.20 0.86* | -0.90 0.84 | -1.70 0.95 |
| Choice reactionTime (msecs) | Placebo | 391.55 12.74 | -1.71 8.86 | -6.82 8.67 | -6.80 8.39 | -6.65 6.85 |
| | 320mg | 386.59 10.15 | -6.48 5.48 | -5.47 5.63 | -1.89 6.10 | -5.40 6.43 |
| | 640mg | 401.91 13.38 | -7.39 8.98 | -26.16 8.42** | -13.74 8.21 | -24.86 8.35** |
| | 960mg | 383.73 10.22 | -5.55 6.50 | -8.44 6.02 | -1.87 6.66 | 1.57 8.24 |
| Spatial Memory (%>chance) | Placebo | 92.56 1.54 | -4.75 3.25 | -1.00 1.64 | -2.13 1.67 | -8.88 2.76 |
| | 320mg | 90.81 4.10 | 1.31 4.32 | -4.19 5.56 | -10.13 4.28 | -3.44 6.09 |
| | 640mg | 85.38 3.79 | 4.44 2.23* | 2.81 4.02 | -1.38 5.59 | -3.88 4.92 |
| | 960mg | 92.69 1.59 | -1.88 2.02 | -3.94 2.00 | -6.31 2.85 | -13.75 5.64 |
| Spatial memory Reaction time (msecs) | Placebo | 556.32 47.74 | -37.53 36.65 | -69.75 41.34 | -39.54 18.40 | -61.00 29.58 |
| | 320mg | 555.26 41.90 | -43.94 29.51 | -56.81 26.97 | -14.50 56.74 | -54.57 23.59 |
| | 640mg | 546.07 30.13 | -17.09 30.05 | -14.03 15.70 | -59.76 16.09 | -39.69 18.08 |
| | 960mg | 524.54 16.77 | -14.90 16.74 | -29.71 11.68 | -22.04 13.96 | -14.15 15.22 |
| NumericWork'g Memory (%>chance) | Placebo | 87.89 1.63 | -1.45 1.86 | -2.78 2.07 | -2.34 2.09 | -1.11 1.92 |
| | 320mg | 87.67 1.92 | -1.22 1.41 | -2.11 1.38 | -3.00 1.42 | -6.00 2.00 |
| | 640mg | 85.67 1.76 | -2.11 1.49 | -5.78 2.85 | -3.56 2.05 | -2.33 1.55 |
| | 960mg | 86.33 2.42 | -2.11 2.34 | 1.00 1.98 | 0.33 2.34 | -3.89 2.20 |
| Numeric Working Memory Reaction Time (msecs) | Placebo | 507.15 18.47 | -5.56 11.57 | -23.75 8.71 | -6.59 11.18 | -26.28 10.35 |
| | 320mg | 513.05 21.93 | -21.36 8.80 | -33.67 11.69 | -20.05 11.07 | -30.08 11.24 |
| | 640mg | 515.80 21.00 | -11.81 11.60 | -17.80 11.52 | -22.64 9.58 | -34.72 11.51 |
| | 960mg | 508.24 18.69 | -21.26 8.73 | -10.60 11.34 | -26.49 9.48*** | -42.79 12.57 |
| Delayed Word Recall (% accuracy) | Placebo | 44.50 3.67 | -12.33 4.61 | -14.83 4.74 | -14.50 4.23 | -17.00 3.62 |
| | 320mg | 39.33 2.80 | -6.50 3.46 | -9.33 3.22 | -4.00 3.90** | -6.33 3.46*** |
| | 640mg | 42.17 4.20 | -12.83 4.06 | -11.50 4.73 | -16.50 5.56 | -14.00 4.32 |
| | 960mg | 39.17 3.15 | -7.17 3.23 | -11.50 3.43 | -11.33 2.83 | -8.17 3.46* |
| Word Recognition (%>chance) | Placebo | 65.00 3.55 | -7.67 4.17 | -11.00 3.82 | -12.00 3.16 | -13.33 4.33 |
| | 320mg | 64.33 4.44 | -9.11 5.21 | -14.67 4.92 | -18.33 6.08 | -17.33 4.52 |
| | 640mg | 61.67 4.69 | -13.33 5.58 | -9.00 4.05 | -15.00 3.96 | -11.33 5.98 |
| | 960mg | 61.33 5.18 | 2.33 5.79 | -13.33 6.14 | -6.33 5.57 | -7.67 5.24 |
| Word Recognition Reaction time (msecs) | Placebo | 622.39 18.95 | 0.00 19.63 | 2.39 22.13 | -7.96 14.44 | 3.30 16.91 |
| | 320mg | 607.07 22.71 | 1.40 18.76 | -9.89 14.52 | 14.40 24.42 | -9.96 15.51 |
| | 640mg | 622.45 23.08 | 19.06 17.38 | -13.60 15.77 | -16.40 12.89 | 3.60 25.34 |
| | 960mg | 629.51 24.42 | 30.60 20.88 | -2.75 15.39 | -9.41 17.00 | -20.18 21.15 |
| Picture Recognition (%>chance) | Placebo | 72.50 4.36 | 1.00 4.65 | -3.75 3.92 | -12.25 9.79 | -11.25 3.92 |
| | 320mg | 77.25 4.60 | -9.75 3.81* | -6.25 4.49 | -8.25 3.76 | -15.75 3.37 |
| | 640mg | 72.75 3.56 | -12.50 3.80** | -8.00 4.22 | -10.00 3.97 | -13.25 4.03 |
| | 960mg | 67.50 5.54 | 4.50 4.07 | 1.25 5.17 | 3.50 4.81*** | 3.00 4.33*** |
| Picture recognit'n Reaction time (msecs) | Placebo | 707.15 25.88 | 14.82 16.40 | -13.38 13.74 | -53.54 43.92 | 0.91 28.51 |
| | 320mg | 692.18 28.32 | 18.80 12.04 | -0.26 15.24 | 7.26 17.60 | -8.38 20.91 |
| | 640mg | 693.66 20.34 | 12.54 14.65 | 27.55 16.32 | 20.06 13.54 | -10.96 13.15 |
| | 960mg | 692.44 20.81 | 20.20 15.24 | 3.17 13.23 | -1.24 15.00 | 3.67 15.52 |

*Figure 4.1. Effects of the ginkgo/ginseng combination on individual task outcome measures from the CDR battery,. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, *****, = 0.0005 compared to placebo)*

### *Secondary Memory Factor*

Performance was enhanced on the 'Secondary Memory' factor for the 960mg dose of the combination at 1 hour [$t(171) = 3.59$; $p = 0.0004$], 4 hours [$t(171) = 2.002$; $p = 0.047$] and 6 hours [$t(171) = 4.23$; $p = 0.0001$] post dose. There was a trend towards improved performance for both the 320mg dose [$t(171) = 1.79$; $p = 0.076$ ] and the 640mg dose [$t(171) = 1.72$; $p = 0.086$]at 6 hours post-dose. Examination of the single task outcome measures making up this factor revealed a number of significant task specific improvements in change from baseline scores, in comparison to placebo. Immediate word recall was significantly improved for all three doses, with 320mg showing improvements at 1 hour [$t(171) = 2.29$; $p = 0.023$], 4 hours[$t(171) = 3.03$; $p = 0.003$ ] and 6 hours post-dose [$t(171) = 3.07$; $p = 0.002$], 640mg resulting in improvements at 2.5 hours [$t(171) = 2.29$; $p = 0.023$], 4 hours [$t(171) = 2.21$; $p = 0.029$ ] and 6 hours post-dose [$t(171) = 2.34$; $p = 0.021$], and 960 mg showing significant improvements at 1 hour [$t(171) = 2.38$; $p = 0.019$], 4 hours [$t(171) = 2.45$; $p = 0.015$] and 6 hours post-dose [$t(171) = 3.16$; $p = 0.002$]. Delayed word recall was also significantly improved following the ingestion of 320mg at 4 hours post-dose [$t(171) = 3.00$; $p = 0.003$] and following both 320 and 960mg at 6 hours post dose ([$t(171) = 3.33$ $p = 0.001$] and [$t(171) = 2.52$; $p = 0.013$] respectively). Accuracy of performance of the delayed picture recognition task was also significantly improved for 960 mg at 4 and 6 hours post-dose ([$t(171) = 3.12$; $p = 0.002$] and [$t(171) = 2.82$; $p = 0.005$] respectively). However at 1 hour post-dose both 320mg and 640mg ([$t(171) = 2.13$; $p = 0.035$] and [$t(171) = 2.67$; $p = 0.008$] respectively) evinced decrements in performance of this measure.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Quality of Memory (%X6) | placebo | 422.79 9.80 | -41.53 11.39 | -47.69 13.18 | -43.61 11.47 | -69.90 9.57 |
| | 320mg | 408.56 9.83 | -28.10 10.94 | -42.71 9.39 | -42.71 12.37 | -50.35 12.15 |
| | 640mg | 393.29 15.64 | -37.67 13.76 | -30.63 13.78 | -49.26 14.02 | -47.79 12.68 |
| | 960mg | 395.52 13.08 | -1.15 13.35 | -33.35 14.73 | -23.15 12.29 | -32.64 15.58 |
| Secondary Memory (%X4) | placebo | 242.33 9.02 | -35.33 9.58 | -43.92 12.28 | -39.54 10.28 | -59.92 9.30 |
| | 320mg | 230.08 7.39 | -28.19 9.82 | -36.42 7.81 | -29.58 10.94 | -40.92 10.05 |
| | 640mg | 222.25 12.60 | -40.00 12.83 | -27.67 11.24 | -44.33 11.61 | -41.58 13.44 |
| | 960mg | 216.50 12.74 | 2.83 13.30 | -30.42 15.22 | -17.17 11.43 | -15.00 11.64 |
| Working Memory (%X2) | placebo | 180.45 2.53 | -6.20 3.72 | -3.78 2.77 | -4.46 2.78 | -9.99 3.48 |
| | 320mg | 178.48 4.08 | 0.09 4.10 | -6.30 5.56 | -13.12 4.66 | -9.44 6.84 |
| | 640mg | 171.04 4.55 | 2.33 2.82 | -2.97 4.85 | -4.93 5.43 | -6.21 4.86 |
| | 960mg | 179.02 3.46 | -3.99 3.65 | -2.94 3.41 | -5.98 3.74 | -17.64 6.83 |
| Speed of Memory (summed msecs) | placebo | 2393.01 86.92 | -28.27 52.41 | -94.49 61.38 | -107.62 47.95 | -83.07 58.08 |
| | 320mg | 2367.56 94.78 | -45.09 49.39 | -100.53 50.74 | -12.88 78.16 | -102.98 32.27 |
| | 640mg | 2377.98 82.38 | 2.71 35.18 | -17.87 33.80 | -78.73 35.59 | -81.78 42.19 |
| | 960mg | 2354.73 69.86 | 14.64 31.59 | -39.89 29.25 | -59.17 25.28 | -73.44 39.30 |
| Quality of Attention (%) | placebo | 90.75 0.59 | 0.35 0.82 | -0.05 0.73 | -0.60 0.85 | -1.00 1.06 |
| | 320mg | 90.90 0.54 | 0.35 0.76 | -0.25 0.55 | -0.20 0.83 | -1.65 1.00 |
| | 640mg | 90.60 0.71 | 0.30 0.89 | -1.30 0.50 | -1.55 0.81 | -2.00 1.14 |
| | 960mg | 91.15 0.55 | -0.15 0.73 | -1.35 0.53 | -0.70 0.90 | -1.45 0.66 |
| Speed of Attention (summed msecs) | placebo | 1033.92 30.97 | 11.41 14.28 | -0.39 15.02 | -3.60 14.63 | 8.01 13.67 |
| | 320mg | 996.82 21.46 | 32.25 14.96 | 27.46 15.92 | 33.84 15.71 | 58.16 16.85 |
| | 640mg | 1037.56 28.76 | 4.11 13.15 | -10.65 13.68 | 24.86 13.35 | 0.32 15.31 |
| | 960mg | 1014.93 26.74 | 4.63 13.22 | -10.83 11.25 | 19.63 14.42 | 10.22 14.81 |

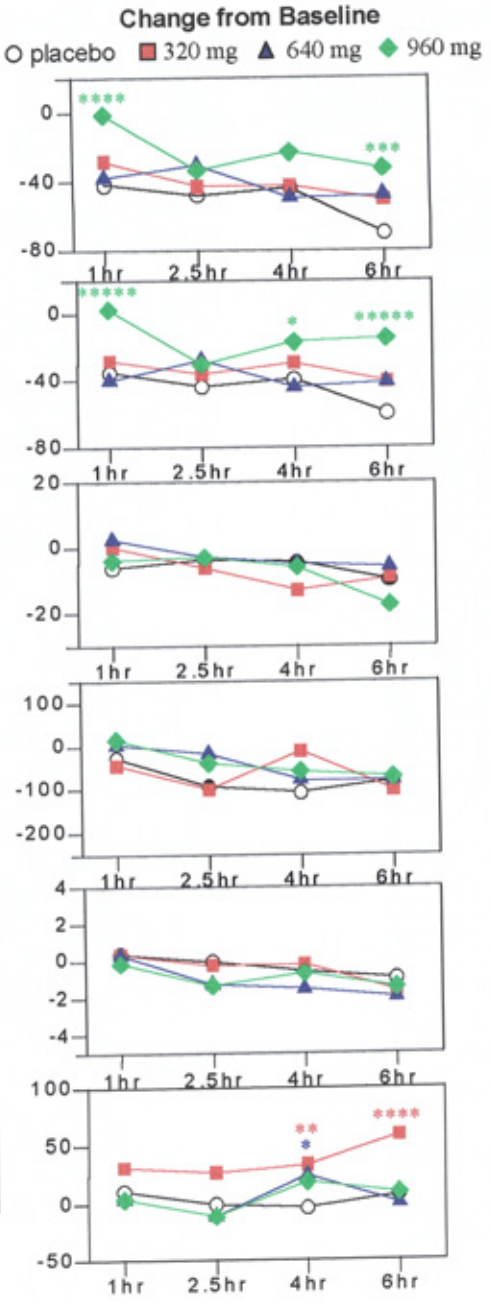


*Figure 4.1. Effects of a Ginkgo biloba/Panax ginseng combination on the cognitive measures;'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Accuracy of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of the combination (320, 640 and 960 mg) and placebo. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01,***, p = 0.005; ****, p = 0.001; *****, p = 0.0005 compared to the corresponding placebo score).Units are as per the table.*

***Working Memory Factor***

There were no significant differences in comparison to placebo on this factor for any of the doses of the ginkgo/ginseng combination at any of the post-dose time points. On the individual task outcomes making up this factor there was a single significant improvement on accuracy of performance of the Spatial memory task following the 640 mg dose at 1 hour post-dose [t(171) = 2.13; p = 0.034).

***Speed of Memory factor***

There were no significant differences in the speed of performance across the memory tasks, but there was a single significant increase in the speed of performance of the numeric working memory task at 4 hours following the ingestion of 960 mg of the combination [t(171) = 3.33; p = 0.001].

***Speed of attention factor***

Speed of performance on this factor was significantly slowed, in comparison to placebo, for both the 320mg and 640mg doses at 4 hours ([*t*(171) = 2.6; p = 0.01] and [*t*(171) = 1.97; p = 0.05] respectively), and for the 320mg dose at 6 hours post dose ([*t*(171) = 3.48; p = 0.0006], There was also a strong trend towards reduced performance for 320 mg at 2.5 hours[*t*(171) =-1.93; *P* = 0.055] Speed was not, however, significantly affected for the 960mg dose of the combination at any time point. This reduction in performance time was reflected in the individual task outcomes with significant slowing of the simple reaction time task following 640mg at 4 hours [t(171) = 1.99; p = 0.048], and on the digit vigilance task at all time points for the 320mg dose of the combination (1 hour [t(171) = 2.78; p = 0.006], 2.5 hours [t(171) = 3.26; p = 0.0013], 4 hours [t(171) = 3.26; p = 0.0013] and 6 hours post-dose [t(171) = 5.25; p = 0.0001], with a similar effect following 640mg at 4 hours post-dose [t(171) = 2.7; p = 0.0076]. However, in contrast to this, speed of performance of the choice reaction time task was

significantly improved at two time points for the 640mg dose - 2.5 hours [t(171) = 2.82; p = 0.0053] and 6 hours post-dose [t(171) = 2.66; p = 0.008].

### *Accuracy of attention factor*

There were no significant differences in performance on the 'Accuracy of Attention' factor. However, decrements on accuracy of performance were observed on the choice reaction time task at 2.5 hours post-dose for both 320 and 960mg ([t(171) = 2.28; p = 0.024] in both cases), and for the 320 mg dose at 6 hours post-dose [t(171) = 2.61; p = 0.01].

### Subjective mood measures

There were no significant differences at any time point for any of the doses of the combination on any of the three mood factors ('alert', 'calm' and 'content') derived from the Bond Lader (Bond and Lader, 1974) visual analogue scales.

### 4.4. Discussion

The results of the current study show that ingestion of single doses of a *Ginkgo biloba/Panax ginseng* combination (*Ginkoba ME*, Pharmaton SA, Switzerland) can affect the cognitive performance of healthy young volunteers in a dose dependent manner.

The most striking effect was the improvement in memory. Performance on the 'Quality of Memory' measure, which comprises accuracy scores for all of the memory tasks in the battery, was enhanced for the highest dose (960mg) at the one hour and six hours post-dose testing sessions. Reference to the measures that comprise this factor showed that whilst the ginkgo/ginseng combination had no discernible effect on the 'Working Memory' factor, the remaining 'Secondary Memory' factor (immediate and delayed word recall, delayed word recognition, delayed picture recognition) evinced a more pronounced effect for the same dose, with stronger improvements at 1 and 6 hours post-dose, and with an additional significant improvement at 2.5 hours post-dose. This effect was accompanied by a trend towards improved performance for both of the other doses at the six hour time point. These observations were further supported by significant single task improvements, most noticeably on the immediate and delayed word recall tasks (Table 4.1). The finding of improvements in memory performance is in direct agreement with previous research showing similar effects following chronic administration of the combination to neurasthenic patients (Wesnes *et al,* 1997), and healthy middle aged participants (Wesnes *et al,* 2000).

In contrast to enhanced performance on memory tasks for the highest dose, the lowest dose of the combination was associated with a decrement at the two later time points (4 and 6 hours) in performance on the 'Speed of Attention' factor, which comprises reaction times from the three tasks assessing attention. The middle dose under investigation was also associated with a significant slowing on this factor at the 4 hour time point.

In Chapter 2 it was found that ingestion of *Ginkgo biloba* was associated with a linear, dose dependent, increase in speed on the 'Speed of Attention' factor for the two highest doses (240

and 360 mg). This was accompanied by an improvement on the 'Quality of Memory' measure that was restricted to two time points for the lowest dose (120 mg) under investigation, but which was largely evident on the 'Secondary' rather than 'Working' memory factor. In contrast to this, in Chapter 3 it was found that *Panax ginseng* administration resulted in a marked improvement in 'Quality of Memory' for the middle dose (400 mg), with this mnemonic effect stronger and evident for all doses when the 'Secondary Memory' factor was isolated. In contrast decrements were evident in 'Speed of Attention' for the less mnemonically active doses (200 and 600 mg). The most notable observation that can be made here is that the pattern of results from the study utilising ginseng alone is strikingly similar to those of the current study. It seems feasible to suggest that similar mechanisms underlie the cognitive effects elicited by both *Panax ginseng* and the *Ginkgo biloba/Panax ginseng* combination. This is not to say, however, that the addition of *Ginkgo biloba* is redundant. The administration of ginseng alone (Chapter 3) resulted in both marked cognitive costs and benefits, with the middle of three doses the most advantageous. In the current case of the combination, whilst one could tentatively suggest that the cognitive effects appear to be more moderate, they are also more readily separable, with cognitive costs largely restricted to the lowest dose, and mnemonic benefits most evident for the highest dose. Perhaps most importantly, no dose of the ginkgo/ginseng combination was associated with the reduction in subjective alertness seen even with the most beneficial dose of ginseng alone. It would appear from the above that the cognitive effects evinced here may be due to a modulatory, rather than additive, antagonistic or synergistic, relationship between the two components. Specifically it appears that the addition of ginkgo to the treatment has tempered the cognitive costs associated with ginseng alone, while preserving the cognitive benefits. This possibility may well be elucidated by an investigation of the comparative effects of the most cognitively beneficial dose of each of the three treatments in a single cohort.

Whilst the pattern of results for ginseng and the combination are somewhat similar on the tasks utilised up to this point, the possibility also exists that the numerous physiological benefits

attributed to *Ginkgo biloba,* and in particular its putative beneficial effect on cerebral blood flow, may well confer an advantage on more 'cognitively demanding' tasks. The question of what the addition of ginseng may bring to performance of such tasks also merits investigation.

# CHAPTER 5. THE COGNITIVE EFFECTS OF ACUTE ADMINISTRATION OF *GINKGO BILOBA, PANAX GINSENG* AND A *GINKGO BILOBA/PANAX GINSENG* COMBINATION: INTERACTION WITH COGNITIVE DEMAND

## 5.1. Introduction

In Chapter 2 the effects of three different single doses of ginkgo (120, 240, 360 mg) were compared with placebo The overall tenor of the results was overwhelmingly positive, with specific linear, dose-dependent improvements throughout the latter three testing sessions on the 'Speed of Attention' factor, comprising reaction time scores from three attentional tasks within the CDR battery. There was also some evidence of improvement in memory performance following the 120 mg dose of ginkgo on the global 'Quality of Memory' measure and for 240 mg on the 'Secondary Memory' factor.

Whilst the specific mechanisms underlying these cognitive effects, and ginkgo's efficacy in treating the symptoms of a number of cerebro-vascular complaints, are unknown, they may be related to the extract's actions as a platelet activating factor antagonist (Braquet and Hosford 1991; Engels and Wirth, 1997), free radical scavenger (Droy-LeFaix, 1997), and modulator of cellular metabolism (e.g. Oberpichler *et al,* 1988). Additionally, ginkgo is capable of *in vitro* modulation of a number of neurotransmitter systems (e.g. White *et al,* 1996, Ramassamy *et al,* 1992). These effects have also been implicated in the demonstration in young volunteers of a reversal of cognitive deficits during hypoxia (Schaffler and Reeh, 1985), and the amelioration of acute mountain sickness (Roncin *et al,* 1996). Such mechanisms may also underlie improvements in cerebral blood flow in rodents (Oberpichler *et al,* 1988), as well as a number of vascular and haematological parameters in humans (Jung *et al,* 1990; Koltringer *et al,* 1993; Roncin *et al,* 1996).

In Chapter 3 the effects of three different single doses of ginseng (200, 400, and 600 mg) were compared with placebo. The results were somewhat mixed, with improvements in 'Secondary Memory' performance that were evident for all doses, but which were most pronounced for the

middle dose under investigation (400 mg). There was, however, also a dose and time dependent slowing of performance on the 'Speed of Attention' factor for the lowest (200 mg) and highest (600 mg) doses of ginseng at the later two post-dose testing sessions (4 and 6 hours). This decrement was accompanied by a significant reduction in subjective ratings of alertness as assessed by Bond-Lader visual analogue scales (Bond and Lader, 1974) for the 200 mg and 400 mg doses at the 6 hour time point.

This demonstration of both cognitive costs and benefits associated with ingestion of ginseng reflects the literature on putative mechanisms. Evidence from *in vitro* and animal studies suggests a plethora of physiological consequences. Ginseng shares with ginkgo a beneficial *in vitro* influence on platelet aggregation (Jung *et al,* 1998; Shi *et al,* 1990), but on the other hand ginseng has also been shown to have opposing effects on a number of parameters including blood pressure (Wood *et al,* 1964), vasoconstriction (Lee *et al,* 1981; Lei and Chiou, 1986), and measures of Hypothalamic-Pituitary-Adrenal axis activity (Luo *et al,* 1993).

These results regarding the acute effects of ginkgo and ginseng may provide valuable insights into the cognitive domains targeted by the extracts. However, the tasks utilised would not be described as being heavily cognitively loaded. It has previously been suggested that a reciprocal relationship exists between the delivery and use of blood-borne metabolic substrates (glucose and oxygen) and the impact of these substrates on cognitive performance (Kennedy and Scholey, 2000; Moss and Scholey, 1996; Scholey *et al,* 1999; Scholey, 2001; Scholey *et al,* 2001). Task performance appears to be 'fuel-limited' under conditions of high cognitive demand, and may be facilitated by any mechanism which serves to aid delivery of glucose and oxygen to active neural tissue. While this model may not hold true for all situations, it does appear to have valuable heuristic value. Thus, administration of oxygen or glucose improves cognitive performance and, particularly in the case of glucose, this effect appears to be more marked under conditions of high cognitive demand (Kennedy and Scholey, 2000; Scholey *et al,* 2001). Moreover, cognitive demand itself can lead to measurable reductions in blood levels of oxygen (Scholey *et al,* 1999) and glucose (Scholey, 2001; Scholey *et al,* 2001).

Typically these investigations have used serial arithmetic tasks where cognitive demand can be systematically titrated. For example, using serial subtractions tasks - where a particular number (usually three or seven) is subtracted from a starting number, then from the resulting number and so on. It has previously been reported that, compared to Serial Threes (repeated subtraction of three), Serial Sevens performance was rated as more demanding, engendered a bigger change in heart rate, was more susceptible to the enhancing effect of glucose and was associated with a greater fall in blood glucose (Kennedy and Scholey, 2000). A version of the computerised Serial Sevens task used in the present study was shown to significantly reduce blood glucose levels compared to a key-pressing control (Scholey *et al*, 2001).

It seems feasible to suggest that more heavily loaded cognitive tasks draw upon blood-borne glucose (and oxygen), and when levels of these substrates are high, facilitation of performance occurs. The evidence suggests that both ginkgo and ginseng affect physiological parameters which may influence the delivery of glucose and oxygen (e.g. blood viscosity, heart rate, blood oxygenation, hypothalamic-pituitary-adrenal axis activity), Whilst this modulation would appear to be beneficial in the case of ginkgo, it is, as yet, unclear as to whether the net effect of ginseng on these parameters is beneficial or detrimental. It therefore seems reasonable to investigate the possibility that these extracts alone and in combination may have differential influences on tasks of differing cognitive load.

In the current Chapter the results from three separate studies, which were undertaken concurrently with the experiments utilising the CDR battery reported in Chapters 2, 3 and 4, are reported. The experiments investigated the effects of the three doses of ginkgo, three doses of ginseng and three doses of their combination on performance of novel computerised versions of the Serial Threes and Serial Sevens tasks.

## 5.2. Materials and Methods

### Participants

Prior to participation in each study participants signed an informed consent form and completed a medical health questionnaire which had been approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Heavy smokers (> 10 cigarettes/day) were excluded from the studies. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning. Eighteen female and 2 male undergraduate volunteers (mean age 19.9 years, SD 1.47) took part in study 1 (*Ginkgo*). In study 2 (*Ginseng*) there were 14 female and 6 male undergraduate volunteers (mean age 21.3 years, SD 2.64). In study 3 which examined the effects of a Ginkgo-Ginseng combination, there were 10 male and 10 female volunteers (mean age 20.6 years, SD 4.2)

### Treatments

***Study 1*** *- Ginkgo biloba:* Treatments comprised of six capsules in which placebos and capsules containing 60 mg *Ginkgo biloba* extract, standardised to a content of 24% *Ginkgo* flavone glycosides and 6% terpene lactones (GK501, Pharmaton SA), were combined to produce a placebo and doses corresponding to 120 mg, 240 mg and 360 mg of the extract.

*Study 2 – Panax ginseng:* On each study day participants received six similar capsules, each containing either 100 mg of ginseng extract (G115, Pharmaton SA), standardised to contain 4% triterpenoid glycosides, or an inert placebo. Depending on the condition to which they were allocated on that particular day the combination corresponded to a dose of either 0 (placebo), 200 mg, 400 mg, or 600 mg of ginseng extract.

*Study 3 – Ginkgo biloba/ Panax ginseng combination:* Treatments were in the form of six capsules of identical appearance, each contained either placebo or a combination of 60 mg of standardised *Ginkgo biloba* extract (GK 501, Pharmaton Switzerland) and 100 mg of standardised *Panax ginseng* extract (G115, Pharmaton Switzerland). Depending on the condition to which they were allocated on that particular day the treatment corresponded to a dose of either 0 (placebo), 320, 640, or 960 mg of the combined extracts.

**Cognitive Measures**

A modified computerised version of the Serial Sevens test was utilised. The original verbal Serial Sevens test (Hayman, 1942) has appeared in a number of forms, including as part of the Mini-Mental State Examination (Folstein *et al,* 1975). It has been used to assess cognitive impairment during hypoglycaemia (e.g. Hale *et al,* 1982; Taylor and Rachman, 1988), and has also been used to investigate the relationship between increased blood glucose levels and cognitive performance (Kennedy and Scholey, 2000; Scholey *et al,* 2001; Scholey, 2001). In the current studies, computerised versions of serial subtractions were implemented (see Scholey *et al,* 2001 for details), here using tests of 2 minutes duration. For the Serial Sevens task a standard instruction screen informed the participant to count backwards in sevens from the given number, as quickly and accurately as possible, using the numeric keypad to enter each response. Participants were also instructed verbally that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of

the first response. Each three-digit response was entered via the numeric keypad with each digit being represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. The task was scored for total number of subtraction and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

The Serial Threes task was identical to Serial Sevens, except that it involved serial subtraction of threes.

**Procedure**

Each participant was required to attend a total of five study days that were conducted seven days apart, to ensure an appropriate wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square which counterbalanced the order of treatments across the four active days of the study. The first day of the study was identical to the following four, but with no treatment (active or placebo). This allowed familiarisation with the tasks and procedure and controlled for practice effects. Data from the first day were not included in the analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment. Further testing sessions took place 1 hour, 2.5 hours, 4 hours and 6 hours following administration of the day's treatment. Each serial subtraction testing session took place after completion of the CDR battery and Bond-Lader visual analogue scales (reported in Chapters 2, 3 and 4) and included the completion of both two-minute computerised subtraction tasks - Serial Threes followed by Serial Sevens.

**Statistics**

In each study the total number of subtraction, and number of error scores were analysed as 'change from baseline' using the Minitab statistical package. The initial analysis was made with a two factor (condition x session) Analysis of Variance with repeated measures on both factors. Following the recommendations of Keppel (1991) the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three treatments at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

## 5.3. Results

### Baseline scores

Prior to analysis of change from baseline data for each experiment, mean pre-dose raw baseline scores for all four conditions (placebo, and the three active conditions) were subjected to a one-way, repeated-measures, Analysis of Variance. There were no significant differences in baseline performance on any of these measures for any of the treatments.

### Study 1 - *Ginkgo biloba*

There were a number of significant time and dose-specific changes following each active dose of ginkgo. Baseline scores, change from baseline scores at each post-dose testing session, and a graphical representation of the latter are represented in Figure 5.1.

Planned comparisons of the change from baseline data revealed that ingestion of all three doses of ginkgo resulted in a significant increase in the number of subtractions made during the Serial Threes task, in comparison to placebo, at the 4 hour testing session. This effect was evident following 120 mg [$t(171) = 2.21$, $p = 0.028$], 240 mg [$t(171) = 4.5$, $p = 0.00001$] and 360 mg [$t(171) = 2.8$, $p = 0.006$], with a single increase at the 6 hour testing session for the 240 mg dose [$t(171) = 2.37$, $p = 0.019$]. Significantly more errors were made following ingestion of 120 mg of *Ginkgo* at the 4 hour testing session [$t(171) = 2.8$, $p = 0.006$].

For Serial Sevens, whilst there were no significant differences in the total number of subtractions for any of the doses of ginkgo, there was a significant improvement in the number of errors in comparison to placebo for all doses at the 2.5 hour time point following 120 mg [$t(171) = 2.16$, $p = 0.032$], 240 mg [$t(171) = 1.98$, $p = 0.049$] and 360 mg [$t(171) = 1.98$, $p = 0.049$]. However, this result can most parsimoniously be attributed to a sharp variation in errors for the placebo condition at this point.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Serial 3s Responses | *placebo* | 52.60 (3.32) | 5.65 (1.98) | 5.60 (1.83) | 3.40 (1.64) | 6.05 (2.05) |
| | *120 mg* | 50.85 (3.81) | 5.90 (1.69) | 7.70 (1.79) | 7.50 (1.37) | 7.55 (1.12) |
| | *240 mg* | 48.55 (5.19) | 5.45 (2.03) | 6.05 (2.44) | 11.75 (1.31) | 10.45 (1.51) |
| | *360 mg* | 50.85 (4.74) | 3.95 (1.69) | 6.15 (2.09) | 8.60 (2.37) | 9.15 (2.19) |
| Serial 3s Errors | *placebo* | 2.75 (0.61) | 0.80 (0.79) | 0.65 (0.73) | -0.15 (0.61) | -0.25 (0.65) |
| | *120 mg* | 1.40 (0.44) | 0.30 (0.23) | 0.90 (0.54) | 1.50 (0.61) | 0.55 (0.59) |
| | *240 mg* | 1.75 (0.43) | -0.15 (0.33) | 0.40 (0.49) | 0.20 (0.53) | 0.05 (0.55) |
| | *360 mg* | 2.35 (0.51) | -0.05 (0.58) | -0.05 (0.55) | 0.85 (0.67) | 0.25 (0.81) |
| Serial 7s Responses | *placebo* | 29.05 (2.10) | 1.75 (1.59) | 3.55 (1.47) | 2.00 (0.93) | 2.95 (1.11) |
| | *120 mg* | 29.10 (2.02) | 2.80 (1.28) | 3.25 (1.47) | 2.65 (1.50) | 1.85 (1.46) |
| | *240 mg* | 30.70 (3.13) | 1.60 (0.89) | 3.25 (1.30) | 3.05 (1.24) | 3.05 (1.44) |
| | *360 mg* | 29.55 (3.14) | 1.90 (0.99) | 3.20 (0.90) | 2.90 (0.70) | 2.60 (1.34) |
| Serial 7s Errors | *placebo* | 2.35 (0.46) | 0.00 (0.45) | 0.75 (0.70) | -0.30 (0.51) | -0.55 (0.49) |
| | *120 mg* | 2.50 (0.47) | -0.60 (0.54) | -0.45 (0.46) | -0.15 (0.36) | -0.65 (0.52) |
| | *240 mg* | 2.20 (0.53) | -0.10 (0.72) | -0.35 (0.64) | -0.85 (0.47) | 0.25 (0.65) |
| | *360 mg* | 2.65 (0.59) | -0.15 (0.54) | -0.35 (0.49) | -0.60 (0.56) | 0.20 (0.71) |

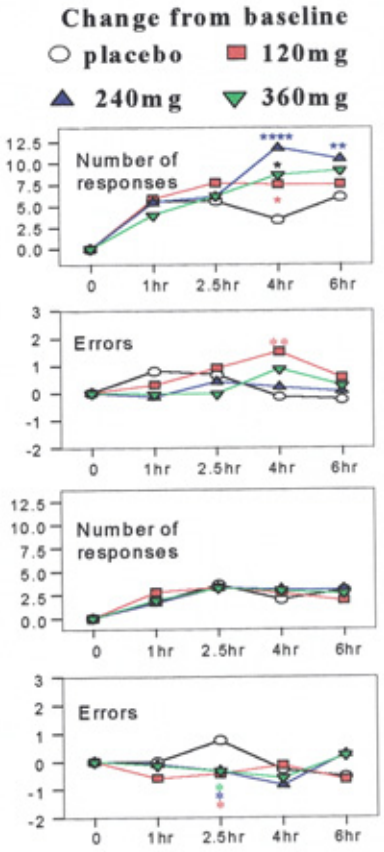


*Figure 5.1 Effects of Ginkgo biloba on Serial Subtractions performance. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of ginkgo. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; ***, p = 0.005; ****, p = 0.001 compared to the corresponding placebo score).*

**Study 2 – *Panax ginseng***

There were a number of significant changes following ginseng administration that were restricted to performance of the Serial Sevens task. Baseline scores, change from baseline scores at each post-dose testing session, and a graphical representation of the latter are represented in Figure 5.2.

Planned comparisons of the change from baseline data from the Serial Threes task revealed that there were no significant differences in comparison to placebo in either total number of subtractions or number of errors for any of the doses of ginseng. In contrast, analysis of Serial Sevens performance revealed a significant decrement in performance for the 200 mg dose of ginseng with participants making fewer subtractions compared with placebo during the 1 hour session [$t(171) = 2.07$, $p = 0.04$], 2.5 hour session [$t(171) = 2.01$, $p = 0.046$] and the 6 hour session [$t(171) = 2.59$, $p = 0.01$]. However, there was a significant improvement in accuracy following the 400 mg dose of ginseng with a reduction in errors, in comparison to placebo, at the 4 hour session [$t(171) = 2.46$, $p = 0.015$], and the 6 hour session [$t(171) = 2.12$, $p = 0.035$], with a similar improvement for the 200 mg dose at the 4 hour time point [$t(171) = 2.01$, $p = 0.046$].

**Study 3 – *Ginkgo biloba/ Panax ginseng* combination**

There were a number of highly significant and sustained improvements in performance following the ginkgo-ginseng combination. Baseline scores, change from baseline scores at each post-dose testing session, and a graphical representation of the latter are represented in Figure 5.3.

Planned comparisons of the change from baseline data revealed that participants made more subtractions in comparison to placebo during the Serial Threes task at the 4 hour testing session following ingestion of 320 mg of the gnkgo/gnseng combination [$t(171) = 2.29$, $p = 0.023$].

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Serial 3s Responses | *placebo* | 62.75 *4.48* | 5.75 *1.54* | 6.80 *1.32* | 6.90 *1.64* | 5.90 *1.77* |
| | *200mg* | 61.70 *3.96* | 4.90 *1.71* | 6.25 *1.72* | 5.40 *2.32* | 5.25 *3.08* |
| | *400mg* | 61.95 *4.51* | 4.60 *1.27* | 5.50 *1.37* | 4.95 *2.05* | 5.40 *1.94* |
| | *600mg* | 60.90 *5.08* | 5.80 *1.67* | 6.35 *1.85* | 7.55 *1.67* | 4.30 *2.33* |
| Serial 3s Errors | *placebo* | 1.30 *0.28* | 0.15 *0.46* | 0.05 *0.29* | 0.05 *0.33* | -0.20 *0.44* |
| | *200mg* | 1.45 *0.37* | 0.90 *0.49* | -0.10 *0.35* | -0.25 *0.39* | 0.30 *0.60* |
| | *400mg* | 1.80 *0.49* | -0.35 *0.27* | -0.05 *0.43* | -0.50 *0.47* | -0.45 *0.41* |
| | *600mg* | 1.35 *0.33* | 0.20 *0.44* | 0.25 *0.49* | 0.35 *0.33* | 0.00 *0.35* |
| Serial 7s Responses | *placebo* | 34.85 *2.34* | 3.80 *1.32* | 4.75 *1.17* | 3.05 *1.56* | 4.80 *1.16* |
| | *200mg* | 35.20 *2.62* | 1.80 *1.01* | 2.80 *1.27* | 1.75 *1.00* | 2.30 *1.31* |
| | *400mg* | 35.80 *3.18* | 2.35 *1.19* | 4.30 *1.11* | 3.50 *1.12* | 4.40 *0.90* |
| | *600mg* | 36.05 *3.18* | 3.55 *1.07* | 4.45 *1.15* | 3.65 *1.30* | 3.10 *0.89* |
| Serial 7s Errors | *placebo* | 1.35 *0.29* | 0.90 *0.46* | 0.05 *0.37* | 0.25 *0.29* | 0.20 *0.29* |
| | *200mg* | 1.90 *0.46* | 0.10 *0.49* | -0.70 *0.50* | -0.65 *0.48* | -0.35 *0.50* |
| | *400mg* | 1.95 *0.42* | 0.10 *0.50* | 0.00 *0.25* | -0.85 *0.38* | -0.75 *0.45* |
| | *600mg* | 1.50 *0.39* | 0.15 *0.37* | 0.15 *0.47* | 0.00 *0.36* | 0.90 *0.62* |

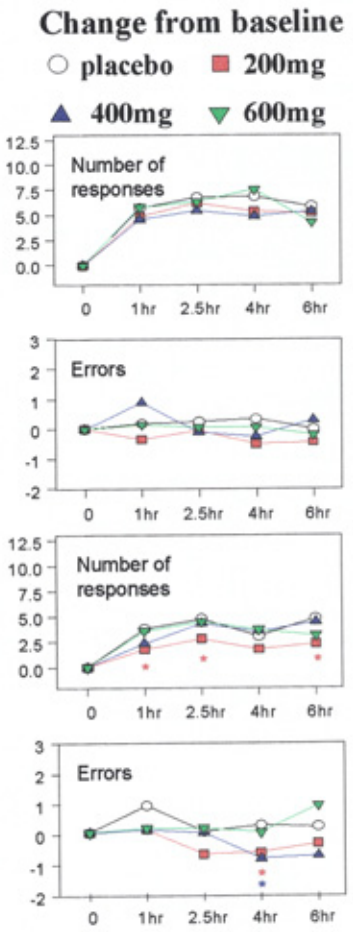


*Figure 5.2 Effects of Panax ginseng on Serial Subtractions performance. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of ginseng. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05 compared to the corresponding placebo score).*

Participants also performed more accurately on Serial Threes. Significantly fewer errors were made during the 2.5 hour session for the 640 mg dose [t(171) = 2.02, p=0.045]. Additionally, error scores were significantly reduced at all time points following the 960 mg dose of the combination. An effect, which was evident by 1 hour post treatment [t(171) = 3.86, p = 0.0001], and maintained at 2.5 hours [t(171) = 2.41, p = 0.017], 4 hours [t(171) = 2.32, p = 0.022] and 6 hours [t(171) = 2.61, p = 0.01].

Moving to Serial Sevens, analysis of the change from baseline data revealed that participants generated significantly more subtractions in comparison to placebo at all time points following the 320 mg dose of the combination. Again this effects was evident by 1 hour post-treatment [t(171) = 3.42, p = 0.0007], and was sustained over the sessions taking place at 2.5 hours [t(171) = 3.36, p = 0.001], 4 hours [t(171) = 3.17, p = 0.002] and 6 hours [t(171) = 2.91, p = 0.004]). Participants also made more subtractions at the 4 hour session following ingestion of 640 mg of the combination [t(171) = 2.91, p = 0.004].

There were also marked improvements in accuracy for all doses of the combination at the 2.5 hour testing session (320 mg [t(171) = 2.22, p = 0.028], 640 mg [t(171) = 3.46, p = 0.0007], 960 mg [t(171) = 3.43, p = 0.0008]), although it should be noted that the sharp increase in errors following placebo was out of keeping with the general pattern of errors for that condition. However, accuracy was also comparatively improved for all doses at the 6 hour session (320 mg [t(171) = 2.02, p = 0.045], 640 mg [t(171) = 3.36, p = 0.001], 960 mg [t(171) = 2.42, p = 0.017], with a single significant reduction at the 4 hour session following the 640 mg dose [t(171) = 2.75, p = 0.007].

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| **Serial 3s Responses** | *placebo* | 66.40 *4.30* | 2.70 *2.27* | 3.40 *1.73* | 3.70 *2.01* | 4.50 *2.00* |
| | *320 mg* | 66.45 *4.94* | 2.35 *1.91* | 5.70 *3.03* | 7.65 *2.25* | 4.85 *2.41* |
| | *640 mg* | 67.50 *4.81* | 3.45 *1.11* | 5.95 *1.25* | 5.00 *1.67* | 6.20 *1.53* |
| | *960 mg* | 64.55 *4.33* | 4.20 *1.71* | 5.15 *1.76* | 6.25 *1.99* | 6.15 *2.36* |
| **Serial 3s Errors** | *placebo* | 1.55 *0.43* | 0.70 *0.51* | 0.70 *0.43* | 0.80 *0.56* | 0.20 *0.39* |
| | *320 mg* | 2.25 *0.49* | 0.05 *0.34* | -0.15 *0.39* | -0.20 *0.47* | 0.80 *0.73* |
| | *640 mg* | 2.45 *0.58* | 0.40 *0.44* | -0.35 *0.56* | -0.05 *0.61* | -0.60 *0.54* |
| | *960 mg* | 2.40 *0.55* | -1.30 *0.55* | -0.55 *0.48* | -0.40 *0.72* | -1.15 *0.57* |
| **Serial 7s Responses** | *placebo* | 39.80 *3.32* | -0.05 *1.81* | 0.85 *1.45* | 0.85 *1.92* | 1.60 *1.71* |
| | *320 mg* | 37.85 *3.39* | 4.60 *1.49* | 5.40 *1.39* | 5.15 *1.39* | 5.55 *1.88* |
| | *640 mg* | 38.45 *3.30* | 2.40 *1.76* | 3.50 *1.46* | 4.80 *1.73* | 3.90 *1.70* |
| | *960 mg* | 38.75 *4.25* | 0.55 *1.46* | 0.70 *1.37* | 2.00 *1.96* | 1.35 *1.90* |
| **Serial 7s Errors** | *placebo* | 2.30 *0.76* | 0.10 *0.70* | 2.05 *1.28* | 0.85 *0.53* | 2.05 *0.63* |
| | *320 mg* | 2.95 *0.53* | 0.85 *0.66* | 0.40 *0.78* | 0.95 *0.76* | 0.55 *0.64* |
| | *640 mg* | 3.40 *0.54* | -0.20 *0.61* | -0.60 *0.56* | -1.20 *0.60* | -0.45 *0.49* |
| | *960 mg* | 2.60 *0.53* | 0.30 *0.47* | -0.50 *0.37* | -0.35 *0.67* | 0.25 *0.73* |

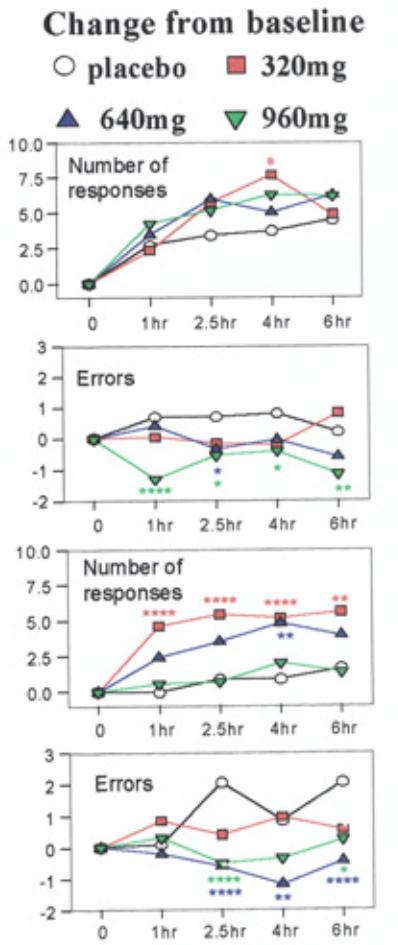


*Figure 5.3 Effects of a Ginkgo biloba/Panax ginseng combination on Serial Subtractions performance. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of the combination. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001 compared to the corresponding placebo score).*

## 5.4. Discussion

Each of the three treatments under investigation significantly affected performance on different aspects of the serial subtraction tasks. The effects of single doses of ginkgo and ginseng were reasonably consistent with previous findings. The most striking result, however, was a marked and sustained improvement in Serial Sevens performance following the ginkgo/ginseng combination.

The demonstration of time and dose dependent increases in the total number of subtractions performed on the Serial Threes task following single doses of ginkgo is broadly in line with the results reported in Chapter 2. Using the more comprehensive CDR test battery, faster 'Speed of Attention' was associated with both a 240 mg and 360 mg dose of ginkgo This modulation was, however, most marked for the higher dose (Chapter 2). Any intervention that produces faster reaction times on attentional measures might reasonably be expected to also result in more responses during a relatively under-loaded task such as Serial Threes. In this case it was the 240 mg dose, rather than the 360 mg dose, that was the most effective in improving performance on the measures used here. Although all three active doses were effective in increasing the number of Serial Three responses at the 4 hour time point, it seems possible that with regards the 120 mg, and possibly even the 240 mg, doses this result could perhaps be most parsimoniously attributed to poor performance at that time point by the placebo condition (Fig 5.1).

Ginseng had no effect on Serial Threes, but was associated with a significant dose specific (200 mg) decrement in total number of subtractions on the Serial Sevens task, which was evident throughout the post-treatment sessions (with the exception of the 4 hour session). There was a reduction in errors for the same dose, which reached significance at the 4 hour session. This pattern hints at the possibility of a time-specific 'speed/accuracy trade-off' for this dose alone, with greater accuracy associated with slower performance. Interestingly the 400 mg dose

produced a specific beneficial effect, evincing a significant reduction in errors at both the 4 and 6 hour testing sessions on Serial Sevens, with a total subtraction performance that remained virtually indistinguishable from placebo (Fig 5.2). Again this pattern is not unlike that gleaned using other measures (Chapter 3), where 400 mg of ginseng was associated with an improved 'Quality of Memory' while the 200 mg and 600 mg doses resulted in a slowing of attentional measures coupled with reduced self-rated alertness at later time points for the 200mg (and 400 mg) dose.

While these results are not inconsistent with the earlier findings (Chapter 2, 3), it should be emphasised that we previously found no effect on a 'Working Memory' factor (comprising accuracy scores from a Numeric working memory and a Spatial working memory task). Serial Subtractions combines features of both attention and working memory, as well as a procedural learning component for Serial Threes (see later). Additionally, unlike the CDR battery tasks used previously, the present tasks were deliberately designed to increase cognitive demand. Therefore as well as similarities between the present study and those which have used the CDR factors, there are also important differences.

Moving to the ginkgo/ginseng combination, performance on the Serial Threes task was improved by all three doses utilised (Fig 5.3). For the lowest dose (320 mg) this improvement took the form of a significant time and dose dependent increase in number of subtractions during the 4 hour testing session. Additionally, significantly improved accuracy was observed at 2.5 hours only for the 640 mg dose, and at all post-dose testing sessions for the 960 mg dose. A similar, but stronger, pattern of results was evinced for the more demanding Serial Sevens task. In this case the 320 mg dose was associated with significantly faster speed of performance sustained across all time points, with a similar (though largely not significant) pattern of improvements for the 640 mg dose. Remarkably, all three doses were also associated with significantly improved accuracy of performance, with this effect being most consistent for the 640 mg dose. Whilst for the 320 mg dose this reduction in errors might be due to a seemingly

chance fluctuation in accuracy for the placebo condition at the 2.5 hours (and possibly 6 hours) testing sessions, it certainly argues against a speed accuracy trade off for either of the most effective doses in terms of number of responses (320 mg) or error reduction (640 mg).

It is clear that this effect of the ginkgo/ginseng combination could not be predicted by simple extrapolation of effects of the two extracts in isolation, either on the tasks used here or previously (Chapter 2, 3). Interestingly, medical herbalism emphasises synergism between components of plant extracts. It would appear that the comprehensive improvements in performance associated with the combination product represent a synergistic behavioural effect of the two extracts.

The mechanisms underlying these effects are not known. In the case of ginkgo there is evidence of beneficial modulatory effects on various vascular and haematological parameters (Koltringer *et al*, 1993; Jung *et al*, 1990; Roncin *et al*, 1996), including cerebral glucose consumption (Rapin *et al*, 1986). Such processes may contribute to facilitated performance on serial subtraction tasks, which are known to be sensitive to simple increase in the delivery of glucose and oxygen (Kennedy and Scholey, 2000; Scholey *et al*, 2001). In contrast ginseng apparently exerts equivocal or even opposite effects on the same parameters (Lee *et al*, 1981; Lei and Chiou, 1986; Wood *et al*, 1964), and both *Panax ginseng* and close family member *Panax quinquefolius* have been shown to engender reduced blood glucose levels, in the case of the former following chronic administration to non-insulin dependent diabetics (Sotaniemi *et al*, 1995), and in the case of the latter during a glucose challenge following acute administration to healthy volunteers and diabetic patients (Vuksan *et al*, 2000a; 2000b; 2001). The extent to which such processes contribute to the effects observed here and elsewhere clearly needs further experimental elucidation.

It has been proposed that the performance of cognitively demanding tasks is to some degree 'fuel limited' by availability of glucose and oxygen (Kennedy and Scholey, 2000; Scholey,

2001; Scholey *et al,* 2001). To what extent might the current findings be accommodated within such a model? Firstly it is not clear as to why Serial Sevens performance was largely unaffected by ginkgo, and secondly why ginseng engendered slower performance on the Serial Sevens. On the first of these points it may be that the implementation of a computerised version of the two tasks has subtly changed their nature. In practice, compared with the verbal version, Serial Sevens appears largely unchanged, with an inherent cognitive demand due to the relatively high working memory and attentional load. On the other hand, compared with the verbal form, computerised Serial Threes performance benefits from the standard 'three-by-three' layout of the computer keyboard numeric keypad. As the task progresses the final digit of each response proceeds down the number pad columns. In essence, Serial Threes could best be described as an attentional task with a procedural learning element. As such the task requires concentration rather than the central executive resources which may be drawn upon during Serial Sevens. This would further support the contention that these results should be viewed in the light of improved 'Speed of Attention' following the same single doses of ginkgo (Chapter 2). Similarly, in Chapter 3 it was demonstrated that single doses of ginseng were associated with a significant slowing of performance on the same attentional factor (in addition to improved memory performance). This effect was accompanied by a decrease in self-rated alertness on Bond-Lader visual analogue scales, which was most marked for the later sessions following the 200 mg dose that was shown to engender the slowing of performance in the current study. Given the plethora of putative physiological effects attributable to ginseng administration, the mechanism, or more likely mechanisms, underlying these effects remain unknown. However it seems unlikely that the opposite effects demonstrated for ginkgo and ginseng on the Serial Subtraction tasks and on the previously reported 'Speed of Attention' factor (Chapters 2,3) are due to opposing influences on the same neural mechanism.

As well as the vascular parameters outlined above, administration of ginkgo is known to modulate a number of neurotransmitter systems (Ramassamy *et al,* 1992; White *et al,* 1996)

including the cholinergic system. Acetylcholine is critically involved in the modulation of attention (Rusted and Warburton, 1991) and ACh synthesis may itself be influenced by availability of glucose (e.g. Kennedy and Scholey, 2000; Messier and Gagnon, 1996; Wenk, 1989) and oxygen (Moss *et al,* 1998; Moss and Scholey, 1996; Scholey *et al,* 1998; 1999). Thus the differential effects of ginkgo and the ginkgo/ginseng combination may be sub-served either through cholinergic mechanisms (Serial Threes), with associated effects on attentional capacity, or through the delivery of metabolic substrates, particularly during high local neuronal demand (Serial Sevens).

Clearly such putative mechanisms are merely conjecture at present. However, such a model may also account for the striking pattern of results following administration of the combination product. In this instance total subtraction performance on the Serial Threes task was similar to that following ginkgo (compare figures 5.1 and 5.3). On the more demanding Serial Sevens task the 320 mg dose was associated with a marked and substantial increase in subtractions across all time points, whilst the other doses engendered improved accuracy. It remains a possibility that the Serial Threes task was improved within the bounds of increased acetylcholine synthesis, whilst only the more demanding Serial Sevens task engendered enough cognitive demand to take advantage of an increased supply of metabolic substrates.

The possibility that these results are the consequence of a synergistic increase in cerebral blood flow coupled with neurotransmitter effects is necessarily highly speculative. Nevertheless it is clear that a number of neurotransmitter systems, metabolic processes and other physiological responses are affected by both ginkgo and ginseng. It seems possible that the cognitively loaded Serial Sevens task may have benefited from a serendipitous combination of these factors interacting with task demands. The extent to which this finding may generalise to other tasks and other populations merits further investigation.

# CHAPTER 6. THE EFFECTS OF *PANAX GINSENG* ON BLOOD GLUCOSE LEVELS AND THE PERFORMANCE OF GLUCOSE SENSITIVE TASKS IN HEALTHY VOLUNTEERS

## 6.1. Introduction

Previous research has suggested that the administration of various members of the *Panax* genus can lead to reduced blood glucose levels in resting normal (Martinez and Staba, 1984; Oshima *et al,* 1987) and alloxan diabetic rodents (Kimura *et al,* 1981; 1999).

A number of studies have also demonstrated reductions in blood glucose levels in both diabetic and non-diabetic humans following ingestion of *Panax quinquefolius* (American Ginseng). Examples include a cross-over experiment, involving 9 participants with Type 2 Diabetes Mellitus, which demonstrated reduced blood glucose levels following a 25g glucose challenge, in comparison to placebo, after ingestion of 3g of powdered American ginseng root (Vuksan *et al,* 2000a). Reductions in blood glucose levels were also evident during a 25g glucose challenge in 10 diabetic patients following ingestion of 3g, 6g, and 9g of powdered American ginseng root (Vuksan *et al,* 2000b). In both cases these reductions were evident irrespective of whether the ginseng was administered prior to (40 mins and 40, 80 and 120 mins in the respective studies) or concurrently with the glucose drink. Similar reductions have also been shown in 10 healthy, non-diabetic volunteers administered 3g of ginseng root 40 minutes prior to a 25g glucose drink (Vuksan *et al,* 2000a), and in 12 non-diabetic volunteers administered 1g, 2g and 3g of powdered American ginseng root 40 minutes prior to the glucose challenge (Vuksan *et al,* 2001). In both cases this effect was either abolished or reduced by concurrent administration of both ginseng and glucose.

One previous study has also looked at the effects of 8 weeks administration of *Panax ginseng,* or placebo, to 36 participants with Type 2 Diabetes Mellitus. Both 100mg and 200mg of

ginseng extract per day led to reductions in fasted blood glucose levels, with the larger dose also improving other physiological indices, including glycated haemoglobin levels (Sotaniemi *et al,* 1995).

Previous research has demonstrated, on one hand, transient cognitive impairment as a result of both hypoglycaemia (e.g. Gold *et al,* 1985; Holmes *et al,* 1984), and lowered but supra-hypoglycaemic glucose levels (e.g. De Feo *et al,* 1988; Taylor and Rachman, 1988), and, on the other hand, improvements in performance on a number of tasks following augmentation of blood glucose levels. In the case of the latter it has been suggested that in healthy young adults instances of cognitive enhancement are most pronounced in the performance of declarative memory tasks (Foster *et al,* 1998), and tasks, or parts of tasks, associated with a relatively high cognitive load (Donohoe, 1997; Kennedy and Scholey, 2000; Sunram-Lea and Foster, 2002). One example of this latter class of tasks is the Serial 7s subtraction task. Performance on a verbal version of the task has been shown to be impaired by reduced blood glucose levels (Hale *et al,* 1982;Taylor and Rachman, 1988), and both verbal (Kennedy and Scholey, 2000) and computerised versions (Scholey *et al,* 2001) of the task have been shown to be improved by augmented blood glucose levels. In the case of both of the latter studies the comparatively less demanding Serial 3s subtraction task was unaffected by the glucose level manipulation.

In Chapter 5 it was suggested that *Ginkgo biloba,* which has been shown to improve both a number of vascular and haematological parameters (Jung *et al,* 1990; Koltringer *et al,* 1993; Roncin *et al,* 1996), and cerebral blood flow (Oberpichler *et al,* 1988), might improve performance on the demanding Serial 7s task by simply increasing delivery of metabolic substrates, including glucose, to the brain. It was also noted that the case for such an effect for ginseng was unclear, with some evidence of a beneficial influence on platelet aggregation (Shi *et al,* 1990; Jung *et al,* 1998), but opposing effects demonstrated on a number of other physiological parameters (Lee *et al,* 1981; Lei and Chiou, 1986; Luo *et al,* 1993; Wood *et al,* 1964). The results from Chapter 5 showed that the 200 mg dose of ginseng resulted in reduced performance on the demanding Serial 7s task, a finding that was broadly in line with decreased

speed on attentional tasks and reduced alertness following the same dose (Chapter 3). Whilst this decrement in performance might be as a result of any of a number of, as yet unidentified, physiological mechanisms, it remains a possibility that it is due to a treatment related reduction in blood glucose levels.

In light of the above, the present study was undertaken to investigate the possibility that single doses of *Panax ginseng* (200 mg and 400 mg), administered to healthy young volunteers, would lead to a reduction in blood glucose levels, both at rest and following a 25g glucose challenge. Performance on a Stroop task, Verbal fluency task, and both easy and hard versions of two serial subtraction tasks was assessed concurrently.

## 6.2.Materials and Methods

### Participants

10 female and 5 male undergraduate volunteers (mean age 18.73 years, range 18-22 years) took part in the study that was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. No smokers took part in the study. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning. Participants consumed their normal breakfast on each day of the study, completing the meal no later than 8am, and consumed no further food or calorific drinks until the 25g glucose challenge at the end of testing.

### Treatments and Glucose Drink

On each study day participants received four capsules of identical appearance, each containing either an inert placebo or 100 mg *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland) standardised to a content of 4% Ginsenosides. Depending on the condition to which they were allocated (counterbalanced Latin square) on that particular day, the combination corresponded to a dose of either 0 (placebo), 200 mg, or 400 mg of *Panax ginseng* extract.

On each day of the study participants received a glucose drink consisting of 25g of glucose powder dissolved in 250ml water and 25ml Robinson Low Calorie fruit squash.

**Blood Glucose Monitoring**

Measurements of blood glucose levels were made using the Cygnus Glucowatch. This device straps to the participants forearm and provides frequent, automatic, and non-invasive glucose measurements by extracting glucose through intact skin via reverse iontophoresis, which is detected by an amperometric biosensor. The Glucowatch received approval for use by diabetics both in this country and the USA in 2001, and has been shown to be accurate and reliable in comparison to standard invasive blood glucose measurements (e.g. Tamada *et al,* 1999; Tierney *et al,* 2001 ).

Following a three hours and 20 minutes calibration period the Glucowatch was set with a single capillary blood glucose reading using a Medisense Exatech Blood Glucose Sensor and disposable MediSense Blood Glucose Test Strip (MediSense Britain Ltd, Birmingham UK). The accuracy and consistency of the MediSense blood glucose monitor has previously been established (e.g. Mathews *et al,* 1987). Following calibration the Glucowatch potentially produces a reading every 20 minutes. Each reading represents averaged and analysed readings taken over two 3 minute periods (minutes 0-3 and 10-13 during each 20 minutes). Due to the sensitivity of the measurement technique the Glucowatch can fail to produce a reading in up to 33% of instances (due to perspiration, physical perturbation, change in temperature, or sharp deviations in glucose levels). In order to maximise the number of readings, participants were required to remain seated in a temperate laboratory, undertaking light leisure activity (videos, reading, etc) throughout the glucose monitoring period.

**Cognitive Measures**

Pre and post dose cognitive tasks were identical, with the exception of the verbal fluency stimuli letters. Task descriptions and running order are described below.

***Verbal Fluency***

Subjects were required to write down as many words as possible beginning with a given letter in one minute. Numbers, proper nouns and repeated prefix's/suffix's were excluded. A version of the task, previously used by Donohoe and Benton (1999) in an investigation of the cognitive effects of glucose, was utilised. In this version two sets of letters matched for their frequency of use are presented. In the pre-dose assessment session participants responded to the letters C, F and L, each for one minute. In the post-dose assessment session participants responded to the letters P, R and W, each for one minute.

***Stroop Task (congruent/incongruent trials)***

Participants responded to the colour of the font (red, blue, green, yellow) in which the words 'red', 'blue', 'green', and 'yellow' were presented, in random order, on a colour monitor. Participants pressed one of four colour coded keyboard keys (F1-F4) as quickly as possible. Two trials were undertaken; 'congruent', in which the presentation colour and meaning of 24 stimuli colour words were the same (e.g. the word 'red' presented in a red font), and 'incongruent', in which the presentation colour and meaning of 24 stimuli words were different (e.g. the word 'red' presented in a green font). The tasks were scored as average reaction time and number incorrect.

***Serial 3s Task***

The participant serially subtracted 3 from a given number, and then the resulting number, for 2 minutes, using a computer number pad. For a full description of both the Serial 3s and 7s tasks see Chapter 5 (pages 141-142). The task was scored as total number of responses and number of errors.

*Serial 7s Task*

As per Serial 3s, but subtracting 7. For task details see Chapter 5 (pages 141-142).

*Easy Random Serial Subtraction Task*

Standardised instructions were presented on the computer screen. A random starting number between 800 and 999, which was cleared by the entry of the first response, was presented on the left hand side of the computer screen. A further number, appeared on the right hand side of the screen, and had to be subtracted from the random starting number. Following each response the right hand number varied randomly between 1 and 4, with this number having to be subtracted from the previous response. Each three-digit response was entered via the numeric keypad with each digit being represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. The task was scored as time taken to complete 24 subtractions, and number of errors. In the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

*Hard Random Serial Subtraction Task*

This was identical to the preceding 'easy' version, with the exception that the numbers on the right of the screen randomly varied between 6 and 9.

**Procedure**

Testing was undertaken in groups of 3-5 participants. The Glucowatches were activated at 9am, after which the participants completed the day's pre-dose baseline cognitive battery. Following the 3 hours and 20 minutes calibration period, and the single capillary blood glucose measurement, glucose monitoring commenced at 12.20pm. The relevant day's treatment, as

dictated by random allocation to a Latin square counterbalancing the treatments over the three days of the trial, was consumed immediately following the first two readings (12.40pm, 1pm). Six resting glucose measurements were then taken (1.20pm. 1.40 pm, 2pm, 2.20pm, 2.40 pm, 3pm), with the 20 minute post-dose cognitive battery being completed during the last measurement period. Following completion of the cognitive battery participants consumed a 25g glucose drink. Two further glucose readings followed (3.20pm, 3.40pm). Due to the loss of single data points glucose readings were averaged across a baseline epoch (average of the calibration reading and the first two potential Glucowatch readings), and two periods representing the average of 3 potential readings during the 0-60 minutes, and 60-120 minute post-dose epochs. The two readings following the glucose drink were considered singly.

**Statistics**

Cognitive outcome change from baseline scores for the placebo condition and each of the two ginseng conditions were analysed with a one factor (condition), within-subjects Analysis of Variance.

Due to a number of missing data points the Analysis of Variance was eschewed for the blood glucose data in favour of paired Bonferroni t-tests (placebo versus each of the two ginseng conditions during each of the two measurement epochs) of the surviving mutual data from each condition. The number of surviving data points are indicated in Table 6.1.

## 6.3. Results

### Blood glucose levels

| Condition | Pre-dose Baseline (mmol/l) | | Change from Baseline (mmol/l) 0-60 minutes | | 60-120 minutes | |
|---|---|---|---|---|---|---|
| *Placebo* | **4.505** | *0.22* | **-0.031** | $0.13^{n=14}$ | **0.173** | $0.20^{n=14}$ |
| *200 mg* | **4.809** | *0.36* | **-0.135** | $0.17^{n=13}$ | **-0.512** | $0.21^{n=14}$ |
| *400 mg* | **4.693** | *0.24* | **-0.076** | $0.16^{n=15}$ | **-0.021** | $0.19^{n=15}$ |

*Table 6.1. Mean blood glucose levels (mmol/l), showing standard errors (italics), and number of observations included, at the pre-dose baseline (average of 3 potential pre-dose readings per participant), and change from baseline blood glucose levels during the time periods 0-60 minutes, and 60-120 minutes.*

#### *Resting blood glucose levels*

Baseline blood glucose readings and change from baseline readings during the two measurement epochs (0-60 and 60-120 minutes) are presented in Table 8.1. Bonferroni t test comparisons of change from baseline data for each active treatment against the corresponding placebo data during each of the two post-dose measurement epochs showed that blood glucose measurements during the 60-120 minute post-dose measurement period were significantly reduced [$t'(26)=2.71$, $P<0.05$] following 200 mg of ginseng, in comparison to placebo.. There were no other significant differences. Mean change from baseline post-dose blood glucose levels are presented graphically in Figure 6.1.

#### *Glucose challenge blood glucose levels*

Following the 25g glucose drink the Glucowatches failed to make a sizeable proportion (>40%) of the expected readings, presumably due to the rapid change in glucose levels. The number of mutual data points between the two ginseng conditions and placebo was reduced to a level that would make any comparison meaningless. In light of this the post-drink data was omitted from further consideration.

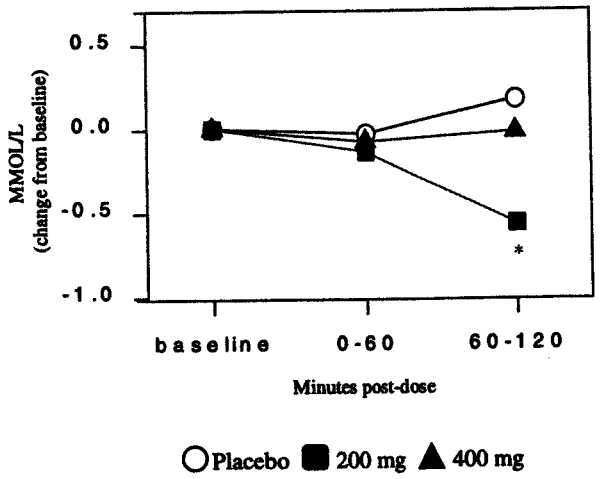


Figure 6.1. 'change from baseline' mean blood glucose levels (mmol/l) during the time periods 0-60 minutes, and 60-120 minutes post-dose (average of the 3 potential readings per participant per epoch) following placebo, 200 mg Ginseng, and 400 mg Ginseng.(* = P<0.05).

## Cognitive outcomes

The ANOVA showed that there were no significant differences in performance between conditions on any of the cognitive measures. Mean baseline and post-treatment scores, and change from baseline scores, with standard errors *(italics)* for each measure are shown in Table 6.2.

| Task | Condition | Pre-treatment | | Post-treatment | | 'Pre-post' treatment change in performance | |
|---|---|---|---|---|---|---|---|
| **Verbal Fluency** (No of Words) | *Placebo* | **44.53** | *2.58* | **44.80** | *0.02* | **0.27** | *1.18* |
| | *200 mg* | **40.87** | *3.79* | **42.13** | *0.02* | **1.27** | *1.42* |
| | *400 mg* | **44.60** | *1.81* | **46.33** | *0.02* | **1.73** | *1.53* |
| **Stroop Congruent** (Seconds) | *Placebo* | **0.51** | *0.04* | **0.46** | *0.02* | **-0.05** | *0.02* |
| | *200 mg* | **0.48** | *0.03* | **0.45** | *0.02* | **-0.03** | *0.01* |
| | *400 mg* | **0.47** | *0.02* | **0.47** | *0.02* | **0.00** | *0.01* |
| **Stroop Incongruent** (Seconds) | *Placebo* | **0.60** | *0.06* | **0.57** | *0.04* | **-0.03** | *0.03* |
| | *200 mg* | **0.60** | *0.05* | **0.57** | *0.03* | **-0.02** | *0.03* |
| | *400 mg* | **0.58** | *0.03* | **0.57** | *0.04* | **-0.01** | *0.02* |
| **Serial 3s Subtraction** (responses) | *Placebo* | **63.57** | *8.46* | **67.53** | *7.15* | **4.21** | *3.06* |
| | *200 mg* | **61.40** | *8.51* | **66.40** | *8.63* | **5.00** | *1.76* |
| | *400 mg* | **64.64** | *8.26* | **67.36** | *7.32* | **2.71** | *2.06* |
| **Errors** (Number) | *Placebo* | **5.43** | *1.24* | **5.33** | *1.27* | **-0.29** | *1.08* |
| | *200 mg* | **5.33** | *1.05* | **5.33** | *0.98* | **0.00** | *1.25* |
| | *400 mg* | **5.29** | *0.83* | **6.71** | *1.34* | **1.43** | *1.47* |
| **Serial 7s Subtraction** (responses) | *Placebo* | **41.73** | *6.73* | **40.87** | *5.71* | **-0.87** | *4.58* |
| | *200 mg* | **40.07** | *6.76* | **37.53** | *5.92* | **-2.53** | *3.15* |
| | *400 mg* | **37.27** | *5.66* | **44.67** | *6.94* | **7.40** | *3.27* |
| **Errors** (Number) | *Placebo* | **5.20** | *0.95* | **5.67** | *1.40* | **0.47** | *0.95* |
| | *200 mg* | **9.07** | *3.63* | **8.53** | *3.35* | **-0.53** | *1.06* |
| | *400 mg* | **4.87** | *0.74* | **4.73** | *0.80* | **-0.13** | *0.80* |
| **Easy random Subtractions** (Seconds) | *Placebo* | **72.23** | *6.45* | **70.59** | *6.18* | **-2.75** | *1.99* |
| | *200 mg* | **77.17** | *8.70* | **67.63** | *6.66* | **-9.54** | *4.48* |
| | *400 mg* | **71.44** | *6.36* | **71.83** | *6.19* | **0.39** | *2.65* |
| **Errors** (Number) | *Placebo* | **1.62** | *0.42* | **1.20** | *0.43* | **-0.38** | *0.60* |
| | *200 mg* | **1.00** | *0.28* | **1.27** | *0.28* | **0.27** | *0.32* |
| | *400 mg* | **1.00** | *0.27* | **1.64** | *0.67* | **0.64** | *0.60* |
| **Hard random Subtractions** (Seconds) | *Placebo* | **129.23** | *17.32* | **121.26** | *13.67* | **-8.70** | *6.41* |
| | *200 mg* | **127.36** | *18.82* | **118.01** | *13.47* | **-9.35** | *8.01* |
| | *400 mg* | **121.63** | *15.66* | **108.09** | *10.93* | **-13.55** | *5.90* |
| **Errors** (Number) | *Placebo* | **3.31** | *0.65* | **2.86** | *0.52* | **-0.75** | *0.74* |
| | *200 mg* | **3.60** | *0.91* | **4.40** | *0.94* | **0.80** | *0.96* |
| | *400 mg* | **3.50** | *0.74* | **3.50** | *0.41* | **0.00** | *0.84* |

*Table 6. 2. Pre and post-dose cognitive task mean scores, and change from baseline scores, with standard errors (italics), for the placebo, 200 mg and 400 mg of ginseng conditions.*

## 6.4.Discussion

The results of the current study demonstrate that a single dose of *Panax ginseng* is capable of reducing the resting blood glucose level of healthy young volunteers. This effect was restricted to the 200mg dose under investigation. No significant concomitant modulation of performance on the selection of cognitive tasks was evident.

The findings with regards glucose levels are broadly in line with demonstrations of reduced fasted blood glucose levels in non-insulin dependent diabetics administered either 100 mg or 200 mg of *Panax ginseng* for 8 weeks (Sotaniemi *et al,* 1995). They also receive qualified support from demonstrations of reduced blood glucose levels during a glucose challenge following large single doses (1–3g) of *Panax quinquefolius* administered prior to a glucose drink in healthy volunteers (Vuksan *et al,* 2000a; 2001), or administered either prior to, or concurrently with, the glucose drink in diabetics (Vuksan *et al,* 2000a; 2000b). Unfortunately, technical data capture problems made analysis of the glucose challenge data from the current study impractical.

Whilst the mechanisms for such modulation are as yet un-delineated, research suggests a number of possibilities. For example, a ginseng mediated increase in insulin secretion, and a specific increase in insulin secretion from pancreatic islet cells stimulated by glucose, has been demonstrated in alloxan diabetic mice (Kimura *et al,* 1981). *Panax ginseng* has also been shown to both increase sheep erythrocyte uptake of glucose (Hasegawa *et al,* 1994), and reduce blood glucose levels and increase liver glucose transporter proteins in orally treated normal and hyperglaecemic mice (Ohnishi *et al,* 1996). Interestingly, it has been suggested that nitric oxide is implicated in both glucose stimulated insulin secretion (Spinas *et al,* 1998), and the uptake of glucose by insulin sensitive tissues (Roy *et al,* 1998). These findings fit well with suggestions (Gillis, 1997) that many of the physiological effects of *Panax ginseng* might be related to enhanced synthesis of nitric oxide.

Although the 200 mg dose of ginseng led to the hypothesised reduction in blood glucose levels, the performance of a range of tasks was not significantly affected by either of the doses of ginseng. The Serial 7s task (Kennedy and Scholey, 2000; Scholey *et al*, 2001), Verbal fluency task (Donohoe and Benton, 1999), and Stroop task (Benton *et al*, 1994), have all previously been shown to be sensitive to glucose level manipulations. However, it is entirely possible that ginseng's blood glucose level effect is as a consequence of an unidentified beneficial mechanism, for instance, increased cellular uptake of circulating glucose (e.g. Hasegawa *et al*, 1994), or increased insulin secretion (e.g. Kimura *et al*, 1981), that may well compensate for the small direct reduction in glucose levels seen in the current study. It is also notable that while the results of previous research (Sotaniemi *et al*, 1995, Vuksan *et al*, 2000a, 2000b, 2001) suggested that a crossover experiment with 15 participants would be adequate to delineate modulation of glucose levels (our primary objective), our own calculations indicated that a sample of this size might be below that which is necessary to reliably detect relatively subtle cognitive effects. It is entirely possible that most of the selection of tasks employed here are simply not sensitive to the effects of ginseng. However, the lack of power, which was dictated by purely practical, resource driven, considerations, is a possible explanation for the lack of any significant demonstrations of cognitive modulation

However, even in the absence of a cognitive effect, it is necessary to note that the restriction of the blood glucose level effect to the 200 mg dose is consistent with the previous demonstration of impaired performance on the glucose sensitive Serial 7s task (Chapter 5), and more generally with selective impairment of alertness and speed of performing attentional tasks (Chapter 3) following this dose of ginseng.

The current study represented the first use of the Glucowatch, which received FDA approval in the USA and became available in the UK in 2001, in an experimental setting (other than those concerning the reliability of its performance). On the face of it, a non-invasive glucose level monitor capable of producing a reading every 20 minutes should be a useful tool in investigations of this nature. However, the reality is that the sensitivity of the technology

employed leads to a preponderance of missed data points, which in the current study meant that data from three potential consecutive readings had to be averaged to arrive at an adequate number of mutual data points between conditions for meaningful analysis. A specific inability to cope with marked changes in glucose levels, which seems somewhat unfortunate in a device that is designed for diabetics to self-monitor fluctuations in their blood glucose levels, led to so little data being available after the glucose drink that analysis of the glucose challenge section of the experiment had to be abandoned. In hindsight the use of the traditional multiple 'finger prick' approach might have been somewhat more informative, if less comfortable for the participants.

Notwithstanding the practical methodological limitations of the current study, the results, showing as they do a dose specific reduction in blood glucose levels as a consequence of the administration of a small single dose of *Panax ginseng*, suggest that this is an area that might benefit from further investigation utilising more reliable blood glucose measurement techniques

# CHAPTER 7. ACUTE COGNITIVE EFFECTS OF SINGLE DOSES OF *GINKGO BILOBA, PANAX GINSENG* AND THEIR COMBINATION IN A SINGLE COHORT

## 7.1. Introduction

In Chapters 2, 3 and 4 respectively the dose-dependent cognitive effects of acute administration of *Ginkgo biloba, Panax ginseng,* and a ginkgo/ginseng combination, to healthy young volunteers, were investigated. All three studies shared the same random allocation, double-blind, placebo-controlled, balanced-crossover design, and utilised the CDR cognitive assessment battery, and the cognitive factors that can be derived from it.

The results of the three studies suggested that all of the treatments had some effect on memory performance. In all three studies improvements were evident on a global 'Quality of Memory' measure comprising accuracy data from all of the battery's memory tasks. A more detailed analysis of the ginseng and ginkgo/ginseng combination results showed that these mnemonic improvements were restricted to the 'Secondary Memory' factor. In the case of ginseng (Chapter 3) these improvements extended across all three doses but were most pronounced, and were apparent at all time points, for the middle (400 mg) dose. Following the ginkgo/ginseng combination (Chapter 4) improvements were restricted to a single dose (960 mg) but were evident at three out of four time points. In the case of *Ginkgo biloba* (Chapter 2) mnemonic improvements were restricted to two time points (1 hr and 4 hr) on the 'Quality of Memory' measure for the lowest (120 mg) dose, and a single time point (4 hr) on the 'Secondary Memory' factor for the 240 mg dose, with a concomitant reduction in 'Speed of Memory' in the case of the latter.

What was most notable in the case of the ginkgo study was a marked, linear, dose dependent improvement on the 'Speed of Attention' factor. This effect was evident from the 2.5 hours post-dose testing session onwards. This was in stark contrast to decrements on the same factor

for doses of ginseng and the ginkgo/ginseng combination. In both cases these decrements were restricted to doses that had showed less or no effect in improving memory. So, whilst the most mnemonically active 400 mg dose of ginseng was unimpaired on this factor, both 200 mg and 600 mg showed significant slowing at the latter two time points (4 and 6 hours), and, whilst 960 mg of the ginkgo/ginseng combination was spared any 'Speed of Attention' modulation, the lowest dose was impaired, again at the latter two time points. Interestingly, the two lowest doses of ginseng were also associated with modulation of mood, with a decrease on the alert factor of the Bond-Lader visual analogue scales which reached significance by the 6 hour testing session.

In Chapter 5 three concurrent, but theoretically distinct, experiments were reported separately. In these studies the effect of the three doses of each treatment on performance of computerised 'serial subtraction' mental arithmetic tasks, which have previously been shown to be sensitive to delivery of metabolic substrates (Kennedy and Scholey, 2000; Scholey *et al,* 2001), was also assessed at each time point, with testing taking place following completion of the CDR battery. Both *Ginkgo biloba* and the gingko/ginseng combination were associated with improved performance. In the case of the former, these improvements were restricted to the easier Serial Threes task, whilst all doses of the combination were associated with significant improvements, of varying magnitude, either in terms of number of subtractions or errors, on both Serial Threes and the more difficult Serial Sevens task. Ginseng not only failed to improve performance, but the lowest dose evinced a significant reduction in Serial Sevens responses across three of the four post-dose testing sessions.

In light of the above demonstrations of memory enhancement, and differential modulation of both the speed of performing tasks assessing attention, and performance of serial subtraction tasks, it seemed appropriate to directly investigate the comparative cognitive effects of a single dose of each herbal product. It is also of particular interest to investigate, in a single cohort, the possibility that the addition of *Ginkgo biloba* might potentiate the novel cognitive effects

demonstrated for *Panax ginseng* previously. To this end the current study utilised the most beneficial dose from the three previous CDR battery based studies (Chapter 2, 3, and 4), with the cognitive effects of 360 mg of *Ginkgo biloba* , 400 mg *Panax ginseng*, and 960 mg of a ginkgo/ginseng combination, being assessed and reported in a single study.

The current study combines the methodology from the previous concurrent studies, reporting the results of both the cognitive factors derived from the CDR battery (Chapters 2, 3, 4), and the computerised serial subtraction tasks (Chapter 5).

## 7.2. Materials and Methods

### Participants

15 female and 5 male undergraduate volunteers (mean age 20.6 years, SD 4.2) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Habitual smokers consuming more than 5 cigarettes/day were excluded from the study. Of the 20 participants 3 were light social smokers and they agreed to abstain from smoking on the day of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Cognitive Measures

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive measures were as described in detail previously in Chapter 2 (section 2.2. pages 87-93).

### Subjective mood measure

Bond-Lader Visual Analogue Scales (Bond and Lader 1974) were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

**Serial subtraction tasks**

Modified computerised versions of the Serial Threes and Serial Sevens tasks, as described in detail in Chapter 5 (section 5. pages 141-142) were employed.

**Treatments**

On each study day participants received ten capsules that were of identical appearance on each occasion. The individual capsules contained either an inert placebo, 60 mg of *Ginkgo biloba* extract (GK501, Pharmaton SA, Lugano, Switzerland), 100 mg of *Panax ginseng* extract (G115, Pharmaton SA, Lugano, Switzerland), or 160 mg of the *Ginkgo/Ginseng* combination (60mg *Ginkgo biloba* extract GK501, Pharmaton SA, Switzerland, and 100mg of *Panax ginseng* extract G115, Pharmaton SA, Switzerland). Depending on the condition to which the participant was allocated on that particular day the combination of capsules corresponded to a dose of either 0 (placebo), 360 mg of *Ginkgo biloba*, 400 mg of *Panax ginseng*, or 960 mg of the *Ginkgo biloba/Panax ginseng* combination.

**Procedure**

An identical procedure to that in Chapters 2, 3, and 4 was employed. Each participant was required to attend a total of five study days that were conducted seven days apart, to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment on visits 2 to 5. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader Visual Analogue Scales, the CDR test battery, and finally the Serial 3s and Serial 7s computerised subtraction tasks.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, the individual task outcome measures, Serial 3s and Serial 7s scores, and the three mood outcomes derived from the Bond-Lader visual analogue scales, were analysed as 'change from baseline' using the Minitab statistical package. The initial analysis was made using a two factor (condition x session) Analysis of Variance with repeated measures on both factors. Following the recommendations of Keppel (1991), the omnibus F test was eschewed in favour of planned comparisons which, were made between the placebo and each of the three active treatment conditions (360 mg ginkgo, 400 mg ginseng and 960 mg ginkgo/ginseng comnbination) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

## 7.3. Results

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 360 mg ginkgo, 400 mg ginseng, 960 mg combination) for each of the primary outcome measures ('Quality of Memory' measure, 5 cognitive factors, mood scale scores, serial subtraction scores) were subjected to a one-way, repeated-measures, Analysis of Variance. There were no significant differences in baseline performance on any measure. However there was a trend towards baseline differences on the working memory factor [$F(3,57)=2.58$, $P=0.063$]. Analysis of the two task outcomes that make up this factor showed that this was due to chance differences on the baseline accuracy score for the spatial memory task [$F(3,57)=3.03$, $P=0.037$]. Analysis using Dunnett's test showed that participants significantly under-performed at baseline prior to taking 960 mg of the combination [$t_d(171)=2.69$, $p<0.05$] in comparison to placebo. A trend towards a baseline difference was also evident on the 'content' score of the Bond-Lader mood scales [$F(3,57)=2.59$, $P=0.062$] . Inspection of the mean visual analogue scale scores suggested that participants rated themselves somewhat more 'content' prior to the ingestion of the placebo than the other treatments.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are presented in Table 7.1. Significant results on individual task outcomes are presented in relationship to the overall factor to which they contribute below (memory task results are presented with either 'Secondary' or 'Working' memory).

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| **Immediate Word Recall** *(% accuracy)* | placebo | 48.67 3.69 | 4.00 2.58 | 1.00 2.70 | -4.33 3.42 | -1.17 3.40 |
| | Ginkgo | 44.67 4.79 | 11.33 4.62 | 0.50 4.41 | 2.67 4.52 | 13.50 4.64***** |
| | ginseng | 48.00 3.86 | 4.67 4.18 | 5.33 4.97 | 12.33 6.04***** | 16.83 5.74***** |
| | Gink/gins | 47.67 4.29 | 10.00 5.91 | 8.17 4.86 | 5.17 5.39* | 1.00 4.74 |
| **Simple Reaction time** *(msecs)* | placebo | 260.98 10.69 | -1.41 7.14 | 5.87 8.76 | 10.25 8.99 | 18.98 9.91 |
| | Ginkgo | 258.67 10.93 | 6.69 5.82 | 9.76 3.81 | 16.14 9.12 | 6.55 5.31 |
| | ginseng | 260.73 10.22 | 5.84 6.22 | 6.83 7.35 | 14.06 8.42 | 8.11 8.26 |
| | Gink/gins | 261.22 9.38 | 7.23 5.61 | 9.22 9.85 | 30.58 12.64** | 8.99 6.01 |
| **Digit Vigilance Accuracy** *(%)* | placebo | 97.67 1.21 | -1.33 1.42 | -1.33 1.50 | -2.00 0.98 | -1.33 1.65 |
| | Ginkgo | 97.33 1.84 | 0.00 2.22 | -0.33 1.71 | -2.67 2.02 | -1.67 1.80 |
| | ginseng | 95.67 1.39 | -1.33 1.65 | 0.67 1.52 | -1.33 1.78 | 1.00 1.69 |
| | Gink/gins | 97.00 1.13 | -0.67 1.80 | -1.00 1.62 | 0.67 1.52 | 1.33 1.42 |
| **Digit Vigilance False alarms** *(number)* | placebo | 0.30 0.16 | 0.15 0.23 | 0.20 0.17 | 0.15 0.24 | -0.05 0.20 |
| | Ginkgo | 0.45 0.14 | -0.05 0.18 | -0.20 0.14* | 0.05 0.29 | 0.10 0.14 |
| | ginseng | 0.55 0.17 | -0.10 0.24 | -0.30 0.19** | -0.35 0.21** | -0.30 0.21 |
| | Gink/gins | 0.30 0.13 | 0.05 0.15 | 0.00 0.18 | 0.25 0.23 | 0.00 0.16 |
| **Digit Vigilance Reaction time** *(msecs)* | placebo | 384.30 12.05 | 9.92 8.38 | -1.25 6.74 | 0.42 6.79 | 9.91 7.86 |
| | Ginkgo | 384.79 10.77 | -2.86 8.64 | 13.54 9.33 | 15.52 8.64 | 12.38 9.73 |
| | ginseng | 388.54 12.13 | -2.34 7.26 | 5.59 6.16 | 10.83 7.61 | 9.30 8.51 |
| | Gink/gins | 392.49 13.23 | 9.19 8.12 | -0.57 8.44 | 4.28 8.95 | 5.07 10.74 |
| **Choice reaction time accuracy** *(%)* | placebo | 93.80 1.15 | -0.70 0.86 | -1.80 0.83 | -0.20 0.86 | 0.40 1.15 |
| | Ginkgo | 93.50 0.78 | 0.20 0.83 | -0.20 0.99 | -0.10 1.17 | -0.30 1.36 |
| | ginseng | 91.90 1.34 | 1.40 0.99* | 0.50 1.06* | 1.40 0.93 | -0.40 1.01 |
| | Gink/gins | 93.40 1.38 | 0.30 1.12 | -0.20 1.19 | -2.20 0.95 | -0.90 1.09 |
| **Choice reactionTime** *(msecs)* | placebo | 388.23 11.48 | 3.12 6.75 | 3.42 6.39 | 6.07 8.30 | 7.63 8.76 |
| | Ginkgo | 396.03 12.11 | -6.37 8.08 | -7.29 6.85 | 4.71 8.85 | -5.16 8.32 |
| | ginseng | 388.30 12.22 | -2.90 7.09 | -7.32 7.94 | 9.22 9.60 | 1.54 7.70 |
| | Gink/gins | 386.23 12.18 | 1.17 5.07 | 5.05 5.61 | 11.54 9.15 | 8.82 5.61 |
| **Spatial Memory** (%>chance) | placebo | 91.81 1.64 | -0.50 2.03 | -5.75 4.80 | -4.63 2.34 | -11.63 5.84 |
| | Ginkgo | 88.50 3.49 | 0.19 5.07 | 3.00 3.68 | -0.06 2.50 | -3.81 2.01 |
| | ginseng | 91.19 1.68 | -6.50 3.23 | -3.00 1.89 | -8.94 5.22 | -12.19 6.75 |
| | Gink/gins | 82.00 4.68 | 6.38 4.30 | 1.13 6.08 | 5.44 3.59* | 3.75 5.80** |
| **Spatial memory Reaction time** *(msecs)* | placebo | 502.57 22.15 | 15.86 15.54 | 7.23 10.74 | -18.37 13.25 | 0.56 14.36 |
| | Ginkgo | 520.85 27.57 | -4.21 14.80 | -22.22 12.37 | 3.75 12.86 | -8.52 20.19 |
| | ginseng | 525.14 23.37 | -16.11 12.90 | -32.20 11.39* | -14.42 16.91 | -20.87 18.99 |
| | Gink/gins | 521.72 23.07 | -17.04 16.23 | -4.56 23.84 | -5.12 18.06 | -3.31 19.46 |
| **NumericWork'g Memory** *(%>chance)* | placebo | 80.67 4.02 | 2.45 2.58 | 2.89 3.28 | -0.89 4.46 | 2.45 3.93 |
| | Ginkgo | 86.11 2.39 | -3.00 1.88* | -4.22 1.40*** | -6.89 1.97** | -3.67 2.33** |
| | ginseng | 83.34 2.62 | -1.45 1.67 | -2.67 2.14* | -2.22 2.90 | -4.00 3.13*** |
| | Gink/gins | 79.11 4.19 | 6.44 3.79 | 3.33 2.91 | -0.55 3.52 | 0.55 4.17 |
| **Numeric Working Memory Reaction Time** *(msecs)* | placebo | 490.60 27.66 | -5.62 8.91 | -11.30 11.41 | 2.98 15.35 | -13.05 15.60 |
| | Ginkgo | 489.78 25.24 | 3.17 13.69 | -6.45 11.40 | 19.99 12.81 | -17.07 11.70 |
| | ginseng | 511.31 26.51 | -17.62 15.54 | -30.38 18.18 | -17.59 20.48 | -27.93 16.54 |
| | Gink/gins | 501.54 27.26 | -2.03 11.95 | -16.58 9.80 | -2.80 15.40 | -23.14 11.88 |
| **Delayed Word Recall** *(% accuracy)* | placebo | 37.00 3.54 | -10.83 2.62 | -12.67 2.61 | -10.33 3.71 | -7.00 2.80 |
| | Ginkgo | 30.50 4.33 | 2.50 5.60* | -3.17 3.89 | -5.50 4.12 | 5.33 5.27* |
| | ginseng | 31.83 3.40 | -4.00 4.08 | -1.00 4.30* | -0.17 7.15 | 10.67 4.14*** |
| | Gink/gins | 32.00 3.66 | 5.67 4.68*** | 7.17 5***** | 5.50 5.67*** | -13.00 4.95 |
| **Word Recognition** *(%>chance)* | placebo | 57.00 4.31 | 1.00 5.28 | -9.13 4.80 | -6.34 4.92 | -3.33 4.26 |
| | Ginkgo | 60.67 5.58 | -3.33 5.55 | -2.56 5.48 | -2.34 4.75 | -6.67 4.64 |
| | ginseng | 60.00 5.64 | -5.00 4.38 | -2.92 2.55 | -3.33 3.67 | -8.33 3.42 |
| | Gink/gins | 60.33 4.55 | -1.96 5.92 | 0.33 3.63 | -13.00 4.97 | -8.33 5.82 |
| **Word Recognition Reaction time** *(msecs)* | placebo | 604.45 25.90 | 2.04 11.11 | 50.61 22.83 | 39.41 25.95 | 1.57 17.89 |
| | Ginkgo | 596.55 23.67 | 24.63 14.63 | 44.47 19.18 | 58.69 18.07 | 25.37 15.58 |
| | ginseng | 618.24 26.07 | 4.55 22.73 | 3.43 20.64* | -8.80 20.44** | -4.17 23.97 |
| | Gink/gins | 605.34 27.42 | 28.20 22.11 | 33.39 21.62 | 6.43 18.62 | 7.15 20.35 |
| **Picture Recognition** *(%>chance)* | placebo | 69.00 4.92 | -9.50 4.78 | -4.25 4.91 | -9.75 4.93 | -10.00 3.94 |
| | Ginkgo | 63.00 6.02 | -2.50 4.10 | -0.25 4.57 | -10.25 2.87 | -6.00 4.93 |
| | ginseng | 65.50 5.76 | -3.75 4.10 | -8.50 5.74 | -7.00 5.22 | -15.25 5.28 |
| | Gink/gins | 64.00 6.88 | -2.75 4.81 | -4.00 5.30 | -9.50 4.85 | -9.00 5.35 |
| **Picture recognit'n Reaction time** *(msecs)* | placebo | 684.02 23.44 | -0.96 15.00 | -12.68 21.93 | 29.36 22.92 | -30.62 17.42 |
| | Ginkgo | 671.73 25.07 | 17.94 20.75 | 5.29 17.25 | 25.99 24.75 | 10.77 18.13* |
| | ginseng | 669.11 24.14 | 12.28 17.58 | 12.4 10.89 | 0.66 22.55 | 2.56 22.65 |
| | Gink/gins | 659.45 26.92 | 11.10 13.36 | 23.48 15.14* | 21.03 17.47 | 3.84 20.73 |

*Table 7.1. Effects of Ginkgo biloba (GK501), Panax ginseng (G115), and a ginkgo/ginseng combination on individual task outcome measures from the CDR battery. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, ****,= 0.001; *****,= 0.0005 compared to placebo).*

**Cognitive factor outcome measures**

Mean raw and change from baseline cognitive factor outcome measure scores for each condition across each session are displayed in Figure 7.1.

***Quality of Memory measure***

Planned comparisons revealed significant improvements in the accuracy of memory task performance, in comparison to placebo, for all three treatments. These improvements were restricted to a single post-dose session for both *Ginkgo biloba* (6 hours [$t$ (171) = 2.67; p = 0.008]) and *Panax ginseng* (4 hours [$t$ (171) = 2.45; p = 0.015]. However, it is also noteworthy that there were trends towards improved performance for both treatments (Ginkgo – 1 hour [t (171)=1.69, p =0.09] and 4 hours [t (171)=1.92, p = 0.056] post-dose. Ginseng- 6 hours post dose [t (171)=1.67, p = 0.096]. In comparison to these relatively modest improvements performance was enhanced for the combination product at 1 hour [t (171)=3.37, p p=0.0009], 2.5 hours [t (171)= 4, p =0.00009], and 4 hours post-dose [t (171)=2.66, p =0.0085].

***Secondary Memory Factor***

When results on the 'Secondary Memory' factor were isolated performance enhanced was noted for all three treatments. Ginkgo showed improvements in performance at 1 hour [$t$ (171) = 2.17; p = 0.032] and 6 hours [$t$ (171) = 2.57; p = 0.011], with a trend towards improvement at 2.5 hours [$t$ (171) = 1.82; $P$ = 0.071] post dose. The ginseng condition evinced improvements at 4 hours [t (171) = 3.02; p = 0.0029] and 6 hours [t (171) = 2.36; p = 0.019], with a further trend towards improvement at 2.5 hours post [t (171)=1.67, p =0.097]. The combination was also associated with significant improvements in comparison to placebo at 1 hour [t (171) = 2.44; p = 0.016], and 2.5 hours post-dose [t (171) = 3.41; p = 0.0008], with a trend towards improved

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 2.5 hours | 4 hours | 6 hours |
|---|---|---|---|---|---|---|
| Quality of Memory (%X6) | placebo | 384.15 *15.32* | -13.39 *10.07* | -27.90 *12.78* | -36.27 *15.21* | -30.68 *10.55* |
| | ginkgo | 373.45 *16.36* | 5.19 *13.33* | -6.70 *12.11* | -22.37 *13.20* | -1.31 *12.25* |
| | ginseng | 379.86 *15.02* | -16.03 *10.79* | -12.75 *11.79* | -9.33 *16.23* | -12.27 *13.14* |
| | Gink/gin | 365.11 *19.70* | 23.78 *16.71* | 16.13 *14.02* | -6.95 *14.08* | -25.03 *15.97* |
| Secondary Memory (%X4) | placebo | 211.67 *12.31* | -15.33 *8.68* | -25.04 *8.46* | -30.75 *10.79* | -21.50 *8.71* |
| | ginkgo | 198.83 *14.27* | 8.00 *12.40* | -5.48 *10.72* | -15.42 *11.58* | 6.17 *11.69* |
| | ginseng | 205.33 *12.39* | -8.08 *9.85* | -7.08 *10.78* | 1.83 *14.50* | 3.92 *10.34* |
| | Gink/gin | 204.00 *13.58* | 10.96 *14.00* | 11.67 *10.43* | -11.83 *11.59* | -29.33 *12.38* |
| Working Memory (%X2) | placebo | 172.48 *4.95* | 1.95 *3.58* | -2.86 *7.03* | -5.51 *5.75* | -9.18 *6.49* |
| | ginkgo | 174.61 *5.32* | -2.81 *5.86* | -1.22 *4.44* | -6.95 *3.64* | -7.48 *2.60* |
| | ginseng | 174.52 *3.83* | -7.95 *3.91* | -5.67 *3.26* | -11.16 *7.58* | -16.19 *8.96* |
| | Gink/gin | 161.11 *7.67* | 12.82 *6.13* | 4.46 *7.36* | 4.88 *5.41* | 4.30 *8.40* |
| Speed of Memory (summed msecs) | placebo | 2281.64 *89.45* | 11.32 *34.80* | 33.86 *51.24* | 53.37 *66.47* | -41.54 *53.55* |
| | ginkgo | 2278.91 *85.02* | 41.54 *44.98* | 21.09 *40.88* | 108.42 *49.50* | 10.54 *47.08* |
| | ginseng | 2323.79 *98.12* | -16.90 *51.01* | -46.67 *41.59* | -40.16 *53.59* * | -50.41 *62.42* |
| | Gink/gin | 2288.05 *96.46* | 20.23 *37.49* | 35.74 *44.22* | 19.54 *41.66* | -15.46 *39.53* |
| Quality of Attention (%) | placebo | 90.55 *0.72* | -1.10 *0.83* | -1.70 *0.60* | -1.15 *0.85* | -0.35 *0.73* |
| | ginkgo | 90.10 *0.94* | 0.15 *1.17* | -0.05 *1.09* | -1.30 *1.32* | -1.00 *0.94* |
| | ginseng | 88.45 *1.02* | 0.20 *0.84* | 0.85 *0.89* | 0.45 *1.08* | 0.55 *0.94* |
| | Gink/gin | 90.05 *0.82* | -0.20 *0.81* | -0.55 *0.97* | -1.05 *0.94* | 0.15 *0.92* |
| Speed of Attention (summed msecs) | placebo | 1033.51 *28.64* | 11.63 *12.13* | 8.03 *15.73* | 16.74 *17.96* | 36.52 *20.41* |
| | ginkgo | 1039.49 *30.61* | -2.54 *12.45* | 16.01 *13.93* | 36.38 *19.56* | 13.77 *14.04* |
| | ginseng | 1037.57 *28.53* | 0.59 *12.74* | 5.10 *13.28* | 34.11 *17.94* | 18.94 *17.81* |
| | Gink/gin | 1039.94 *29.02* | 17.60 *14.19* | 13.70 *18.94* | 46.40 *23.80* | 22.87 *14.67* |

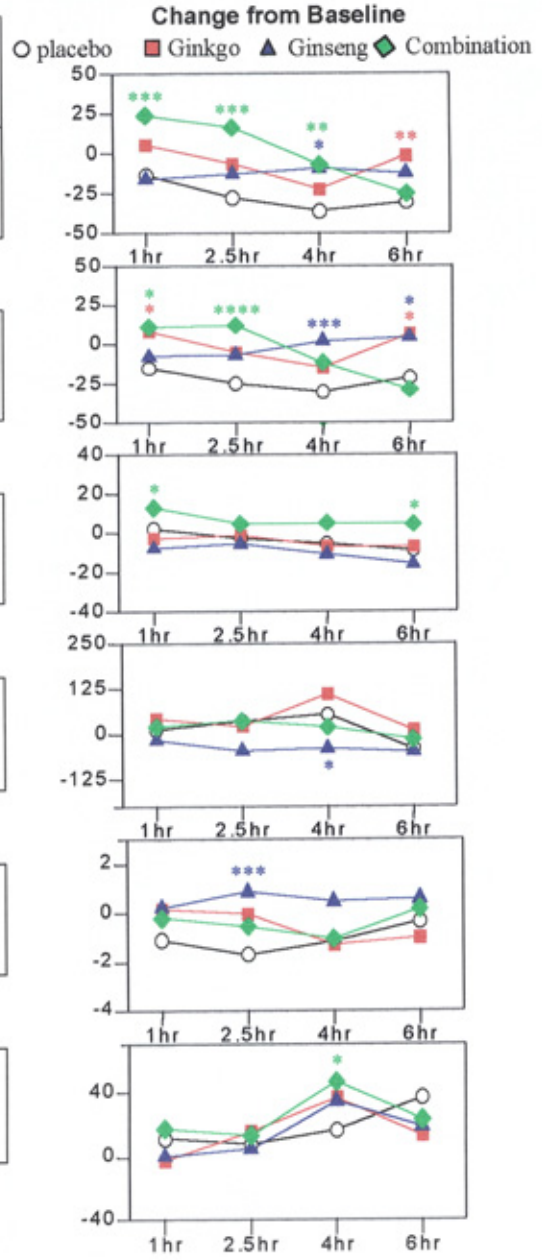


*Figure 7.1. Effects of Ginkgo biloba, Panax ginseng and a ginkgo/ginseng combination on cognitive measures; 'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Accuracy of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of ginkgo. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001 compared to the corresponding placebo score). Units are as per the table.*

performance at 4 hours post-dose [t (171) = 1.76; p = 0.08]. Inspection of the individual task outcomes showed that all three treatments were associated with improved performance on both the immediate and delayed word recall tasks. On the former task participants performed better following administration of ginkgo at 6 hours post-dose [t (171) = 3.56; p = 0.0005], with trends towards improved performance at 1 [t (171) = 1.78; P = 0.08] and 4 hours post-dose [t (171) = 1.7; p = 0.09]. Following ginseng administration participants performed better at 4 hours [t (171) = 4.05; p = 0.00008] and 6 hours post-dose [t (171) = 4.37; p = 0.00002], and following the ginkgo/ginseng combination participants performed better at 4 hours [t (171) = 2.31; p = 0.022] with a trend towards improvement at 2.5 hours post-dose [t (171) = 1.74; p = 0.084]. A similar pattern was evident on the delayed word recall task, with ginkgo showing improvements in comparison to placebo at 1 hour [t (171) = 2.46; p = 0.015] and 6 hours [t (171) = 2.28; p = 0.024], with a trend towards improvement at 2.5 hours post-dose [t (171) = 1.75; p = 0.08], ginseng showing improvements at 2.5 hours [t (171) = 2.15; p = 0.033] and 6 hours [t (171) = 3.26; p = 0.001] with a trend towards improvement at the intermediate 4 hour post-dose testing session [t (171) = 1.88; p = 0.06], and the ginkgo/ginseng combination showing improvements at 1 hour [t(171) = 3.05; p = 0.0027], 2.5 hours [t(171) = 3.66; p = 0.0003] and 4 hours post-dose [t(171) = 2.92; p = 0.003].

***Working Memory Factor***

Performance was enhanced for the combination product at 1 hour [t (171)=2.03, p =0.043] and 6 hours post-dose [t (171)=2.52, p =0.013]. There was also a trend towards improved performance for the same treatment at the 4-hour [t (171)=1.94, p =0.053] testing session.

On the individual task outcomes making up this factor there were significant improvements in accuracy of performance of the spatial memory task following the 960 mg dose of the

combination at 4 hours and 6 hours post-dose ([t (171) = 2.15; p = 0.033) and [t (171) = 3.28; p = 0.001] respectively). In light of the chance significant baseline differences on the spatial memory task, and resultant trend towards a significant difference in baseline performance between conditions on this factor, it seems inappropriate to over interpret the above findings.

However, the two treatments that had not shown any baseline differences in performance were associated with decrements in the accuracy of performance on the numeric working memory task. This effect was evident at all time points for ginkgo (1 hour [*t* (171) = 2.42; p = 0.016], 2.5 hours [t (171)=3.16, P=0.002], 4 hours [*t* (171) = 2.67; p = 0.008] and 6 hours [*t* (171) =2.72; p = 0.007]), and at 2.5 hours [*t* (171) = 2.47; p = 0.014], and 6 hours [*t* (171) = 2.87; p = 0.005] for ginseng.

***Speed of Memory factor***

Participants' change from baseline data reflected consistently faster performance on the 'Speed of Memory' factor throughout the post-dose testing sessions in the ginseng condition. This increase in speed reached significance at 4 hours [*t* (171) = 2.2; p = 0.029] having evinced a trend towards faster performance at 2.5 hours post-dose [*t* (171) = 1.89; p = 0.06].

Inspection of the single task outcome data showed that whilst in the ginseng condition participants performed significantly faster than placebo on both the spatial memory task and the word recognition task at 2.5 hours post-dose ([*t* (171) = 2.47; p = 0.014], and [*t* (171) = 2.32; p = 0.022] respectively), with a further significant improvement on the latter task at 4 hours post-dose [*t* (171) = 2.62; p = 0.001]. It should however be noted that both the combination product (2.5 hours post-dose [*t* (171) = 2.07; p = 0.04]) and ginkgo (6 hours post-dose [*t* (171) = 2.62; p = 0.02]) were associated with a single instance of slower change from baseline performance on the picture recognition task.

*Quality of* **Attention** *factor*

The ginseng condition evinced a single significant improvement on this factor at 2.5 hours post-dose [$t$ (171) = 2.91; p = 0.004], with a trend towards the same at 4 hours post-dose [$t$ (171) = 1.83; p = 0.07]. In line with this, significant change from baseline improvements were seen for this condition on the single tasks, with improved accuracy on the choice reaction time task at 1 hour [$t$ (171) = 2.03; p = 0.044] and 2.5 hours post-dose [$t$ (171) = 2.22; p = 0.028], and reduced false alarms on the digit vigilance task at 2.5 hours and 4 hours post-dose ([$t$ (171) = 2.03; p = 0.044] in both cases). The ginkgo condition was also associated with reduced false alarms on this measure at 2.5 hours post-dose [$t$ (171) = 2.1; p = 0.036].

***Speed of attention factor***

The ginkgo/ginseng combination was associated with a single reduction in speed on this factor at 4 hours post-dose [$t$ (171) = 2.09; p = 0.038]. In line with this, simple reaction time speed was also slowed at the same time point [$t$ (171) = 2.76; p = 0.006]. There were no other significant differences on either the factor or single tasks.

**Serial Subtraction Tasks**

***Serial Threes Task***

Due to data capture errors the total number of participants contributing scores to the Serial Threes task was reduced to 18. The total number of responses, in terms of change from baseline and in comparison to placebo, on the Serial Threes task was significantly increased in the ginkgo/ginseng condition at 6 hours post-dose [t (153) = 2.69; p = 0.008].

Participants also made less errors on the task 4 hours after ingesting *Ginkgo biloba* [$t$(153) = 2.2; p = 0.029], with a trend towards reduced errors for the same dose at the following (6 hour) time point [$t$(153) = 1.83; p = 0.069].

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Serial 3s Responses | *placebo* | 70.06 *6.13* | 4.44 *1.83* | 6.42 *1.49* | 3.22 *1.78* | 1.72 *3.87* |
| | *Ginkgo* | 68.89 *6.25* | 3.33 *2.80* | 6.59 *2.60* | 6.28 *2.75* | 5.78 *2.58* |
| | *Ginseng* | 70.06 *5.88* | 5.67 *2.02* | 6.06 *1.88* | 3.39 *2.11* | 6.00 *2.59* |
| | *combin* | 68.61 *5.82* | 4.33 *1.94* | 5.78 *2.21* | 6.28 *2.82* | 7.89 *2.63* |
| Serial 3s Errors | *placebo* | 4.17 *0.81* | 0.83 *0.97* | 0.89 *0.78* | 2.22 *0.82* | 1.89 *1.01* |
| | *Ginkgo* | 5.17 *0.88* | 1.33 *1.12* | 0.18 *0.83* | -0.44 *0.91* | -0.33 *1.01* |
| | *Ginseng* | 4.39 *0.55* | -0.28 *0.78* | 0.17 *0.84* | 1.94 *1.12* | 1.44 *1.26* |
| | *combin* | 4.06 *0.75* | 0.67 *1.10* | 0.33 *0.72* | 2.17 *1.60* | 0.61 *1.21* |
| Serial 7s Responses | *placebo* | 43.42 *4.07* | 2.74 *1.13* | 2.68 *0.88* | 0.84 *1.35* | 1.53 *0.98* |
| | *Ginkgo* | 42.28 *4.82* | 5.11 *1.85* | 3.89 *1.24* | 4.21 *1.02* | 6.37 *2.12* |
| | *Ginseng* | 42.82 *3.99* | 2.84 *1.63* | 3.58 *1.39* | 3.00 *1.35* | 3.95 *1.63* |
| | *combin* | 44.56 *4.35* | 1.47 *1.30* | 3.74 *1.33* | 4.32 *1.40* | 2.05 *1.34* |
| Serial 7s Errors | *placebo* | 4.63 *0.80* | -0.05 *0.59* | 0.11 *0.70* | 1.26 *1.15* | 1.32 *0.63* |
| | *Ginkgo* | 4.72 *0.54* | 0.58 *0.66* | 0.47 *0.76* | 0.00 *0.58* | 1.63 *0.88* |
| | *Ginseng* | 5.12 *0.70* | 0.00 *0.58* | -0.11 *0.57* | -0.16 *0.59* | 0.00 *0.67* |
| | *combin* | 4.94 *0.74* | 0.11 *0.75* | 0.68 *0.65* | 0.74 *0.71* | -0.47 *0.46* |

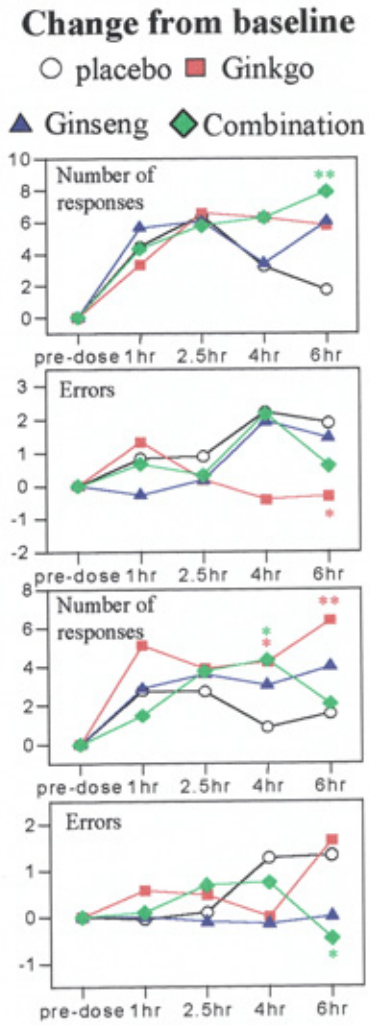


*Figure 7.2. Effects of Ginkgo biloba (GK501), ginseng (G115), and a ginkgo/ginseng combination on serial subtraction performance. The table presents means (with standard errors in italics) of baseline and change from baseline scores for each treatment in terms of both total reponses and errors on both the Serial Threes and Serial Sevens tasks. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01 compared to the corresponding placebo score).*

Mean raw and change from baseline serial subtraction scores for each condition across each session are displayed in Figure 7.2.

***Serial Sevens Task***

Due to data capture errors the total number of participants contributing scores to the Serial Sevens task was reduced to 19.

Participants made more responses on the Serial Sevens task at the testing session 4 hours after taking both *Ginkgo biloba* [$t(162) = 2.29$; $p = 0.023$], and the ginkgo/ginseng combination [$t(162) = 2.36$; $p = 0.019$]. This significant improvement in performance was sustained in the ginkgo condition with more responses at 6 hours post-dose [$t(162) = 3.29$; $p = 0.0012$], and in the ginkgo/ginseng condition with fewer errors at 6 hours post-dose [$t(162) = 2.24$; $p = 0.027$].

**Subjective mood measures**

***'Alert'***

Participants subjective ratings showed that they rated themselves as becoming progressively more alert following ingestion of ginkgo at each time point in terms of change from baseline scores, and in comparison to placebo ( 1 hour [$t(171) = 2.26$; $p = 0.025$], 2.5 hours [$t(171) = 2.28$; $p = 0.024$], 4 hours [$t(171) = 2.86$; $p = 0.005$], and 6 hours post-dose [$t(171) = 2.34$; $p = 0.001$]).

***'Content'***

Participants also became more 'content' than at the baseline testing session, in comparison to placebo, following both ginkgo at 1 hour [$t(171) = 2.82$; $p = 0.005$], 4 hours [$t(171) = 3.5$; $p =$

0.0006] and 6 hours post-dose [$t(171) = 3.34$; $p = 0.001$], and following the ginkgo/ginseng combination at 2.5 hours [$t(171) = 3.12$; $p = 0.002$], 4 hours [$t(171) = 2.59$; $p = 0.01$], and 6 hours post-dose [$t(171) = 3.44$; $p = 0.0007$].

*'Calm'*

There were no significant differences on the 'calm' factor.

Mean raw and change from baseline scores on the 'alert', 'content' and 'calm' factors derived from the Bond-Lader visual analogue scales for each condition across each session are displayed in Figure 7.3.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 2.5 hours | 4 hours | 6 hours |
|---|---|---|---|---|---|---|
| 'Alert' | *placebo* | 55.62 3.84 | 3.74 2.22 | 3.02 4.05 | 2.21 3.73 | 2.41 3.68 |
| | *ginkgo* | 50.72 3.84 | 9.36 2.91 | 8.71 3.78 | 9.36 3.07 | 10.77 3.75 |
| | *ginseng* | 52.53 4.00 | 5.93 2.63 | 6.31 3.53 | 6.68 3.69 | 3.99 3.33 |
| | *Gink/gin* | 55.19 3.86 | 1.43 2.31 | 0.52 1.91 | 3.34 2.36 | 2.53 2.68 |
| 'Calm' | *placebo* | 70.39 3.07 | -1.73 1.84 | -3.91 2.45 | -3.53 3.34 | -4.98 4.19 |
| | *ginkgo* | 62.80 3.80 | 4.74 2.80 | 0.48 2.40 | 4.50 2.51 | 2.69 3.80 |
| | *ginseng* | 65.63 3.14 | 0.41 2.33 | 0.09 2.80 | -0.47 2.79 | -1.90 4.01 |
| | *Gink/gin* | 65.50 4.05 | 1.36 2.92 | 3.25 2.38 | 2.42 3.18 | 2.92 3.01 |
| 'Content' | *placebo* | 59.45 3.65 | 2.38 2.78 | -0.08 3.42 | -1.20 4.46 | 2.23 3.12 |
| | *ginkgo* | 61.85 3.10 | 0.75 2.70 | -2.50 3.01 | -1.58 2.60 | -7.13 3.51 |
| | *ginseng* | 59.90 4.57 | -1.18 2.68 | -2.50 3.60 | 1.38 3.46 | 0.25 2.94 |
| | *Gink/gin* | 60.90 3.38 | 1.18 3.42 | 2.03 4.01 | -0.40 3.88 | -0.60 4.42 |

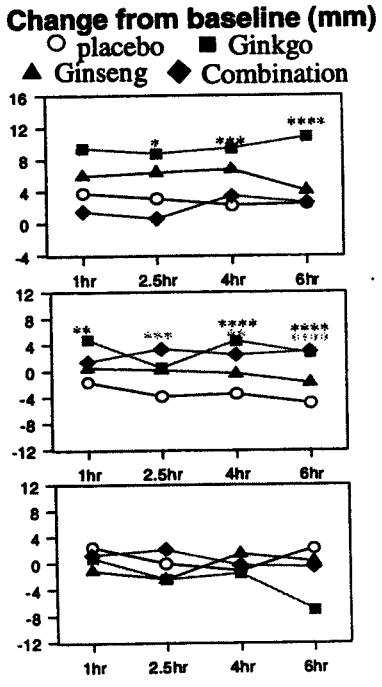


*Figure 7.3. Effects of Ginkgo biloba (GK501), ginseng (G115), and a ginkgo/ginseng combination on Bond-Lader mood scale factor scores; 'Alert', 'Content', and 'Calm'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each treatment. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001 compared to the corresponding placebo).*

## 7.4. Discussion

The results of the current study confirm that ingestion of single doses of *Ginkgo biloba, Panax ginseng*, and a product combining the two extracts can beneficially affect the cognitive performance of healthy young volunteers. The profile of the results is also notable in that it conforms substantially to that generated across the four previous studies (Chapters 2, 3, 4, and 5) looking at the effects of acute doses of each treatment on the performance of the cognitive tests utilised here.

The most striking effect in the current study was the improvement from pre-dose baseline levels in memory performance, in comparison to placebo, evident following administration of all three treatments. Whist this effect was apparent for all doses at a minimum of one time point on the 'Quality of Memory' measure, it was more pronounced on the 'Secondary Memory' sub-factor. Indeed, the improvements seen on the 'Working Memory' factor, which were only apparent following administration of the ginkgo/ginseng combination, can most parsimoniously be ascribed to a regression to the mean following chance under-performance at the pre-dose session on one of the two tasks making up this factor. If this is the case, the demonstrated improvements, restricted as they are to 'Secondary Memory', represent a direct replication of previously reported results for all three herbal preparations (Chapters 2, 3, 4 ), with the single proviso that the dose of ginkgo under investigation here was not one that had been associated with mnemonic improvement in the previous study (Chapter 2).

It is particularly interesting to note that, in contrast to the unexpected mnemonic improvement following the 360 mg dose of ginkgo, the previously demonstrated improvements on the 'Speed of Attention' factor (Chapter 2) were absent. Whilst this suggests that the earlier result might have been a simple anomaly, the clear, linear, dose-dependent nature of the original effect argues against this. Alternatively there may have been minor procedural differences in the two experiments (e.g. additional verbal instructions to the standard on screen instructions which may have led to greater attention being given to accuracy at the expense of speed), also we

cannot rule out a simple cohort effect.. With regards the 'Speed of Attention' factor, it is notable that the dose of ginseng utilised here did not evince the decrements previously associated with other doses of this treatments on this factor (Chapters 3). However, the ginkgo/ginseng combination did engender a significant decrement at a single time point (4 hours), although the overall pattern of modulation on this measure is far from consistent.

With regard to the other factors, whilst not wishing to over-interpret single significant differences, it is notable that ginseng outperformed all of the other conditions at all time points on both the 'Speed of Memory' and 'Accuracy of Attention' factors This effect reached significance at a single time point, with a further trend towards significance at another consecutive time point, in each case. These results confirm the absence of any speed/accuracy trade off on timed memory task performance, and may hint at a more general beneficial cognitive effect of the extract. This possibility is also supported by the absence of the previously observed (Chapter 3) detrimental effect on the 'alert' factor of the Bond-Lader mood scales

With regard to mood, it is also noteworthy that whilst in the *Ginkgo biloba* condition participants subjectively recorded improvements from baseline significantly greater than those in the placebo condition at all time points on the 'alert' factor, and at all time points except 2.5 hours on the 'content' factor. A similar effect was evident on the 'content' factor following the ginkgo/ginseng combination, with improvements at all but the first (1 hour) testing session. Again the results on the 'content' factor should be interpreted with caution, as the pre-dose baseline ANOVA showed a chance trend towards differences in scores, with participants rating themselves most content prior to placebo. Whilst it is possible that the significant differences on this factor may partly reflect a regression to the mean, it seems reasonable to assume that the relationship between subjective ratings made on a single day is more pertinent than that between ratings made on separate days.

Previous findings of modulation of performance on the serial subtraction tasks (Chapter 5) are also substantially supported. In the previous study it was specifically hypothesised that any treatment related improvements in cerebral blood flow, or vascular and haematological parameters, would lead to augmented delivery of metabolic substrates to the brain during cognitive demand. It has previously been suggested that such improvements in energy delivery underlie the preferential improvement of more demanding cognitive tasks, for instance Serial Sevens rather than Serial Threes, following administration of glucose and oxygen (Kennedy and Scholey, 2000; Scholey *et al,* 2001; Scholey, 2001). Such beneficial physiological effects have been attributed to *Ginkgo biloba* (e.g. Jung *et al,* 1990; Koltringer *et al,* 1993; Oberpichler *et al,* 1988; Roncin *et al,* 1996), and the ginkgo/ginseng combination (Kiesewetter *et al,* 1992), but the evidence with regard to ginseng is equivocal (Gillis 1997). Indeed, both *Panax ginseng* (Sotaniemi *et al,* 1995) and family member *Panax quinquefolius* (Vuksan *et al,* 2000) have recently been shown to engender a reduction in circulating blood glucose levels. It was therefore expected that there would be a preferential improvement of Serial Sevens following ginkgo and the ginkgo/ginseng combination, but not following ginseng.

The results of the previous study (Chapter 5) lent some support to this proposition. Whilst ginkgo (most notably 240 mg) only led to increased subtractions on the Serial Threes task during the later sessions, all doses of the ginkgo/ginseng combination (but most notably 320 mg) were associated with improvements, either in accuracy or number of responses, on both tasks, with this most pronounced on the Serial Sevens task. The lowest dose of ginseng, in contrast, was associated with decrements on the number of subtractions on the Serial Sevens task.

Despite the fact that the current study utilised a dose of ginkgo (360 mg) previously shown to be less than optimum for this task, and the least effective dose of the ginkgo/ginseng combination (960 mg), the results that were generated lend further support to the original hypothesis. In the case of the current study, in comparison to placebo, 360 mg of ginkgo was

associated with improved accuracy at 4 hours post-dose on the Serial Threes task, and increased total subtractions during the later testing sessions (4 and 6 hours) on the more demanding Serial Sevens task. The ginkgo/ginseng combination led to increased subtractions at 6 hours post-dose on the Serial Threes task, and improved total number of subtractions and accuracy, at 4 and 6 hours respectively, on the Serial Sevens task. 400 mg of ginseng was once again not associated with any significant modulation on either task.

It is tempting, in light of these results, to tentatively suggest a possible fractionation of cognitive effects similar to that previously proposed for augmented blood glucose levels (Kennedy and Scholey, 2000). i.e. that the improvements on cognitively demanding serial subtraction tasks seen here may be due to a simple physiological augmentation in the delivery of metabolic substrates, whereas the improvements seen across secondary memory tasks may be due to modulation, primarily, of neurotransmitter function or cellular events.

Beyond this straightforward replication of results from the previous studies one of the primary objectives of the current investigation was to make a comparison of the cognitive benefits of ginseng and the ginkgo/ginseng combination in a single cohort. This seemed a particularly pertinent question in light of secondary memory improvements for both treatments, which seemed to be somewhat stronger for ginseng alone (Chapters 3, 4). The results of the current study suggest that on the 'Secondary Memory' factor there is little to choose between ginseng and the product combining it with ginkgo, other than that the combination was associated with significant improvements at the earlier two time points, whilst ginseng alone improved performance at the latter two time points. The other measures employed suggest that both treatments also confer additional, but differing, benefits. In the case of the ginkgo/ginseng combination this takes the form of improved performance on the serial subtraction tasks, with possible beneficial modulation of mood, whilst for ginseng both the speed of performing the timed memory tasks, and accuracy of performing the 'attention' tasks, appear to be beneficially affected. Whilst the extant literature has failed to adequately explore the cognitive effects of

either acute or chronic administration of ginseng, it seems that chronic regimens of both *Ginkgo biloba* and the ginkgo/ginseng combination confer cognitive benefits in a number of populations (e.g. Kanowski *et al,* 1996; Kleijnen and Knipschild, 1992b; Le Bars *et al,* 1997; Wesnes *et al,* 1997; 2000). It would therefore seem timely not only to investigate the effects of chronic regimens of ginseng in cognitively compromised populations, but also to address the question of whether the addition of *Ginkgo biloba*, with its comparatively well delineated benefits across a number of physiological dimensions, provides any additive or synergistic cognition enhancing properties following chronic administration.

# CHAPTER 8. ELECTROENCEPHALOGRAPH (EEG) EFFECTS OF SINGLE DOSES OF *GINKGO BILOBA* AND *PANAX GINSENG*

## 8.1. Introduction

Chronic administration of standardised extracts of *Ginkgo biloba* are reported to ameliorate the cognitive decline associated with ageing (e.g. Allain *et al,* 1993; Rai *et al,* 1991), and attenuate the cognitive deficits associated with intermittent claudication (Draebeck *et al,* 1996), vascular dementia, Alzheimer's disease (Kanowski *et al,* 1996; Le Bars *et al,* 1997), and cerebral insufficiency (Kleijnen and Knipschild, 1992). A number of studies in these populations have demonstrated modulation of Electroencephalograph (EEG) activity. Typically, in the resting, eyes-closed EEG, this has taken the form of either a reduction in theta waveband activity, or a simultaneous decrease in theta and increase in alpha waveband activity. Such a pattern of modulation of relative theta/alpha waveband activity, which has been interpreted both as a 'normalisation' of activity (Itil *et al,* 1996), and indicative of increased vigilance (Geβner *et al,* 1985), has been reported following chronic administration of ginkgo to sufferers from age associated cognitive impairment (Geβner *et al,* 1985; Pidoux *et al,* 1983), cerebral insufficiency (Hofferberth, 1995; Schulz *et al,* 1991) and Alzheimer's disease (Hofferberth 1994). Both acute and chronic administration of 120 mg of a *Ginkgo biloba* extract to sufferers from age associated cognitive impairment has also been reported to shorten P300 latency (Semlitsch *et al,* 1995).

A linear, dose-dependent increase, in comparison to placebo, in the resting, eyes-closed alpha activity (occipital recording) of 12 healthy unimpaired adults has also been reported as a consequence of ingestion of three doses (40 mg, 120 mg, 240 mg) of ginkgo extract (Itil *et al,* 1996). In a further placebo-controlled, acute dose (80 mg and 160 mg) study involving eight testing sessions spanning six hours, Luthinger *et al* (1995) reported increased relative and absolute alpha-1 (8-9.5 Hz) power in frontal regions of the scalp. Relative alpha-2 (10-12.5 Hz) power was also increased frontally, but the absolute power of this band showed a trend towards

a decrease in the same region. Following sub-acute treatment (160 mg for 5 days) a concomitant increase in relative and absolute beta waveband power, and a decrease in relative theta waveband power were also noted. In this study both contingent negative variation (CNV) and P300 amplitude were also investigated, but the pattern of modulation, with both significant increases and decreases over the 8 testing sessions is not easy to interpret. These results, from studies involving healthy cohorts, should also be seen in the light of a previous paper (Kunkel, 1993) that reported the results from two separate, acute dosage, cross-over experiments, and which showed a large number of treatment-related effects. However, there was no clear pattern to the results, with all of the three doses of ginkgo (40, 80 and 160 mg Egb 761), and the two fractions of the extract that were used generating markedly different profiles of significant resting EEG waveband modulation.

The evidence with regards the effects of ginseng is less clear-cut, with little methodologically sound evidence for its efficacy, either in the enhancement of cognition, or treatment of pathological conditions (see Bahrke and Morgan 2000; Vogler *et al,* 1999 for comprehensive overviews). It is noteworthy that no study to date has investigated the EEG effects of ginseng.

The results of Chapters 2 and 3 suggested that whilst both ginkgo and ginseng, but most notably the latter, led to dose dependent/specific enhancement of memory performance, ginkgo was associated with a linear dose-dependent improvement in the speed of performing attention tasks (Chapter 2). In contrast, both the lowest (200 mg) and highest (600 mg) dose of ginseng led to a deterioration in speed across the same tasks (Chapter 3). Similarly, while ginkgo improved the speed of performing computerised mental arithmetic tasks, the lowest dose of ginseng led to reduced speed (Chapter 5). In Chapter 7 the mnemonic effect of 400 mg of ginseng was replicated. However, 360 mg of ginkgo failed to produce a speeding of attentional task performance, but did improve memory performance. The same dose also improved the speed or accuracy of both of the serial subtraction tasks.

In light of this pattern of results it seems timely to investigate the bioelectrical effects of single doses of both *Panax Ginseng* and *Ginkgo biloba*. The current study therefore comprised a double-blind, counterbalanced, cross-over experiment, involving a single cohort of healthy young (<40) volunteers. Both event related potentials and resting EEG recordings were investigated following administration of a placebo and the doses of ginkgo (360 mg) and ginseng (200 mg) seen to both modulate serial subtraction performance, and have the most striking opposite effects on the speed of performing attention tasks. Cognitive performance was assessed using a shortened version of the Cognitive Drug Research (CDR Ltd) computerised assessment battery previously utilised.

## 8.2. Materials and methods

### Participants

10 female and 5 male volunteers (mean age 26.6 years, range 19-39 years) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Habitual smokers consuming more than 5 cigarettes/day were excluded from the study. Of the 15 participants 3 were light social smokers and they agreed to abstain from smoking on the day of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Treatments

On each study day participants received eight capsules that were of identical appearance. The individual capsules contained either placebo, 60 mg of *Ginkgo biloba* extract (GK501, Pharmaton SA, Lugano, Switzerland), or 100 mg of *Panax ginseng* extract (G115 Pharmaton SA, Lugano, Switzerland). Depending on the condition to which the participant was allocated on that particular day the combination of capsules corresponded to a dose of either 0 (placebo), 360 mg *Ginkgo biloba*, or 200 mg *Panax ginseng*.

### Procedure

The study employed a within subjects, double blind, placebo controlled, balanced-crossover design with single-dose administration (placebo, 360 mg *Ginkgo biloba* or 200 mg *Panax*

*ginseng)* of the treatment on the relevant days. Participants attended on three separate occasions and received the treatment in an order dictated by a Latin square. Each day of the study was separated by a 7 day 'wash-out' period. EEG recording took place four hours after ingestion of the day's treatment. The running order of the EEG measures was: contingent negative variation; auditory evoked potentials; 'eyes open' wave band analysis; and 'eyes closed' waveband analysis. A short battery of cognitive tasks comprising the shortened CDR battery and Bond-Lader mood scales were administered following completion of the EEG recording. Details of all tasks and measures are given below.

**EEG Recording**

EEG was recorded from disposable silver/silver chloride gel-filled electrodes attached to the scalp with collodion according to the international 10/20 system. 18 recording sites ($N_Z$, $F_Z$, $C_Z$, $P_Z$, $F_3$, $F_4$, $F_7$, $F_8$, $C_3$, $C_4$, $T_3$, $T_4$, $T_5$, $T_6$, $P_3$, $P_4$, $O_1$, $O_2$) all referred to linked mastoids, were utilised. EEG signals were amplified using a Neuroscan Synamps system (Neurosoft Inc., Sterling, VA, USA). Eye movement compensation was derived from nasion linked-mastoid electrodes. Participants were asked to visually fixate on a small red cross displayed on a monitor in an effort to minimise eye movements during all eyes open EEG recordings Any sections of the EEG recording still contaminated with eye movements, muscular activity or other artefacts were excluded from the analysis.

Amp settings (filter) during the three recording periods were: Contingent Negative Variation – Low Pass 30 Hz, High Pass 0.02 Hz; Auditory Evoked Potentials – Low Pass 30 Hz, High Pass 0.15 Hz; and Power/Frequency Spectrum – Low Pass 100 Hz, High Pass 0.1 Hz.

Event related potentials and wave band analyses were conducted using the Neuroscan 4 Workstation programme (Neurosoft Inc., Sterling, VA, USA).

**Contingent Negative Variation (CNV)**

CNV was elicited with warning tones presented binaurally through earphones (1 KHz, 20ms, 60dB), followed 1.25 milliseconds later by binaural imperative tones (650 Hz, 400ms, 60dB), to which the participant responded with a dominant hand index finger push button.

**Auditory evoked potentials**

P300 amplitude and latency were elicited with a standard auditory oddball paradigm. Common and target tones were presented binaurally through headphones. Participants were instructed to listen for and count 19, 20 or 21 infrequent target tones (650 Hz, 60 dB, 200 ms) which occurred randomly amongst 82 to 90 frequent common non target tones (1 KHz, 60 dB, 200 ms). Interstimulus intervals varied randomly between 1250 ms and 3000 ms.

**Power/Frequency spectrum**

The power/frequency spectrum of the resting EEG was calculated by Fast Fourier transform of the average of 50 two second continuous epochs of resting EEG activity, eyes open and eyes closed, over the frequency range 0.5-26.8 Hz in 0.487 Hz steps. Total power was expressed as $\mu V^2/Hz$ in the delta (0.5-3.9 Hz), theta (4.3-7.8 Hz), alpha (8.2-14.1Hz), and beta (14.6-26.8 Hz) frequency bands. Additionally, given the exploratory nature of this study, further analyses were performed on more restricted frequency bands (see below).

**Cognitive Assessment**

A shortened version of the CDR computerised assessment battery utilised previously in Chapters 2, 3 and 4 was administered.

The selection of computer controlled tasks from the system was administered with parallel forms of the tests being presented at each testing session. The current shortened version retained the outcome measures making up the 'Speed of Attention' and 'Accuracy of Attention'

factors, and three of the four outcome measures making up the 'Secondary Memory' factor (i.e. Picture recognition was omitted). These factors formed the primary cognitive outcome measures. Task details, factor descriptions, and the contribution of the single task outcome to the relevant factor are detailed in Chapter 2 (pages 87-93).

**Subjective mood measure**

The Bond-Lader Visual Analogue Scales (Bond and Lader 1974) were combined as recommended by the authors to form three mood factors representing 'alertness', 'calmness' and 'contentedness'.

**EEG Descriptive Statistical Mapping**

The mean EEG power (obtained from grand means of the participants data following placebo and the relevant treatment) at each frequency interval of 0.487 Hz across the frequency spectrum (0.5 – 26.8 Hz) and at each electrode site were calculated both individually and grouped into the four standard frequency bands (delta, theta, alpha and beta). Paired t test values for each 0.487 Hz interval and each frequency band (eyes open and eyes closed), were mapped over the surface of the head using the Neuroscan 'Window' programme, which is based on a linear interpolation algorithm linking each individual electrode with its four nearest neighbouring electrodes.

**Statistical Analysis**

***EEG and performance data***

Mean P300 latency and amplitude, and mean frequency band power (delta, theta, alpha and beta) were calculated during each treatment (placebo, ginkgo, ginseng). across groups of electrodes representing frontal ($F_Z$, $F_3$, $F_4$, $F_7$, $F_8$,), left temporal ($C_3$, $T_3$, $T_5$,), right temporal ($C_4$,

$T_4$, $T_6$), parietal ( $C_Z$, $P_Z$, $P_3$, $P_4$) and occipital ($O_1$, $O_2$ ) scalp regions. Normality of the data was assessed using the Anderson-Darling test, and measures that deviated from normality were log-transformed to a normal distribution prior to analysis (Gasser *et al,* 1982). Following any necessary transformation, two factor (treatment x scalp region) repeated measures ANOVAs were carried out for each measure. Where appropriate, *post hoc* comparisons of the individual treatment versus placebo for overall condition means were carried out using Dunnett's test. Individual *post-hoc* comparisons of ginkgo versus placebo and ginseng versus placebo within each individual brain region were made using Bonferroni t tests utilising MS error from the Analysis of variance.

One factor (condition) repeated measures ANOVAs were carried out on the cognitive outcome data ('Secondary Memory', 'Speed of Attention' and 'Accuracy of Attention' factors) and on the three mood measures ('alert', 'content', and 'calm') derived from the Bond-Lader mood scales.

***Correlations of EEG and cognitive performance***

In order to examine the possibility of a topographic relationship between modulation of the EEG and cognitive performance Pearson product-moment correlations of change (treatment minus placebo) in cognitive outcome scores against change (treatment minus placebo) in P300 latency and amplitude, and power of the 'eyes closed' frequency bands, were calculated for each electrode site.

## 8.3. Results

### Log Transformation

Data from each of the four wavebands (delta, theta, alpha and beta) were log transformed to a normal distribution prior to Analysis of Variance (Gasser 1982).

### EEG

P300 amplitude and latency, and the mean power (eyes closed) for the frequency bands (delta, theta, alpha and beta), in the individual brain regions, four hours following ingestion of placebo, 360 mg of *Ginkgo biloba* and 200 mg of *Panax ginseng* are presented in Table 8.1.

### CNV

There were no significant differences on the measures of CNV (Data not shown).

### P300

Whilst there were no significant differences on the amplitude of the P300 wave, the Analysis of Variance of P300 latency showed that there was a significant interaction between treatment and scalp regions [$F(8,112) = 2.07$, $p=0.044$). *Post-hoc* comparisons (Dunnett's) showed that the overall latency of the P300 wave was significantly reduced in the ginseng condition ($p < 0.05$). Comparison (Bonferroni t) of each treatment against placebo in the individual scalp regions revealed that ginseng evinced a significant reduction in latency in both the left temporal and occipital groupings of electrodes ($p < 0.01$ in both cases).

### 'Eyes Open' Power/Frequency wavebands

There were no significant differences on the 'eyes open' waveband analysis (data not shown).

| EEG measure | Scalp Region | Placebo | Ginkgo | Ginseng |
|---|---|---|---|---|
| **P300 Amplitude (μV)** | *Frontal* | 7.45 | 7.45 | 6.84 |
| | *Left Temporal* | 10.78 | 9.89 | 9.69 |
| | *Right Temporal* | 10.57 | 10.19 | 9.94 |
| | *Parietal* | 12.87 | 11.93 | 11.44 |
| | *Occipital* | 10.13 | 9.29 | 9.04 |
| **P300 Latency (milliseconds)** | *Frontal* | 339.79 | 339.92 | ***335.35*** |
| | *Left Temporal* | 348.27 | 344.6 | ***334.18**** |
| | *Right Temporal* | 337.11 | 340.24 | ***334.78*** |
| | *Parietal* | 337.62 | 342.64 | ***332.43*** |
| | *Occipital* | 345.2 | 339 | ***333.23**** |

| Waveband | | Mean Power ($\mu V^2$/Hz) 'eyes closed' | | |
|---|---|---|---|---|
| **Delta (0.5-3.9 Hz)** | **Frontal** | 55.09 | 39.69 | 48.65 |
| | *Left Temporal* | 14.37 | 11.44 | 12.91 |
| | *Right Temporal* | 13.32 | 11.93 | 17.08 |
| | *Parietal* | 15.52 | 15.36 | 14.42 |
| | *Occipital* | 12.66 | 11.33 | 11.58 |
| **Theta (4.3-7.8 Hz)** | *Frontal* | 4.46 | 3.35** | ***3.1**** |
| | *Left Temporal* | 3.28 | 3.11 | ***2.87*** |
| | *Right Temporal* | 3.28 | 3.30 | ***2.92*** |
| | *Parietal* | 4.89 | 4.97 | ***4.31*** |
| | *Occipital* | 4.00 | 4.40 | ***3.89*** |
| **Alpha (8.2-14.1Hz)** | *Frontal* | 4.04 | 3.09 | 3.05* |
| | *Left Temporal* | 5.33 | 4.96 | 4.91 |
| | *Right Temporal* | 5.72 | 6.11 | 5.54 |
| | *Parietal* | 9.03 | 8.59 | 8.60 |
| | *Occipital* | 17.66 | 20.37 | 19.79 |
| **Beta (14.6-26.8 Hz)** | *Frontal* | 0.68 | 0.52** | ***0.51**** |
| | *Left Temporal* | 0.76 | 0.67 | ***0.64*** |
| | *Right Temporal* | 0.83 | 0.80 | ***0.67*** |
| | *Parietal* | 0.96 | 0.94 | ***0.87*** |
| | *Occipital* | 1.14 | 1.23 | ***1.08*** |

*Table 8.1. Mean P300 amplitude and latency, and mean 'eyes closed' power in the delta, theta, alpha and beta wavebands for both treatments and placebo, averaged across electrodes grouped into frontal, left temporal, right temporal, parietal and occipital scalp regions. Asterisks denote the significance of post-hoc comparisons (Bonferroni t) of the relevant active treatment mean against placebo in the brain region (* < 0.05, ** < 0.01). Bold italicised means indicate an overall significant difference (Dunnett's test) between the relevant condition and placebo. Analysis of Variance and post-hoc comparisons of the waveband power were undertaken on log-transformed data (not shown).*

## 'Eyes Closed' Power/Frequency wavebands

### *Delta (0.5-3.9 Hz)*

There were no significant differences on the delta waveband.

### *Theta (4.3-7.8 Hz)*

The Analysis of Variance revealed a trend towards a main effect of treatment on the power of the theta waveband [$F(2,28) = 2.93$, $p = 0.07$], and a significant interaction between the treatment (placebo/ginkgo/ginseng) and the brain regions [$F(8,112) = 2.92$, $p = 0.005$]. *Post-*

*hoc* comparisons (Dunnett's) showed that theta activity was reduced overall by ginseng in comparison to placebo ($p < 0.05$). Comparison of placebo against each treatment in individual scalp regions (Bonferroni t) revealed that power was reduced across the frontal electrodes following both ginkgo and ginseng administration (both $p < 0.01$).

***Alpha (8.2-14.1Hz)***

A significant interaction between treatment and brain regions was evident ($F(8,112) = 3.44$, $p = 0.001$). *Post-hoc* comparisons (Bonferroni t) showed that the power of alpha activity across the frontal scalp region was significantly decreased by ginseng ($p < 0.05$).

***Beta (14.6-26.8 Hz)***

A significant interaction between treatment and brain regions was also evident within the beta waveband ($F(8,112) = 2.51$, $p = 0.015$). *Post-hoc* comparisons (Dunnett's) of treatment means against placebo means showed that the power of the beta waveband was reduced overall following ingestion of ginseng ($p < 0.05$). Power was also specifically reduced in the frontal scalp region following both ginkgo ($p < 0.01$), and ginseng administration ($p < 0.01$).

Descriptive topographic probability maps (paired t tests) of each waveband, showing comparative, smoothed reductions in EEG activity following both treatments, in comparison to placebo, are presented in Figure 8.1. (ginkgo versus placebo) and Figure 8.2. (ginseng versus placebo). Both figures include topographic maps showing reductions in activity at every 0.487 Hz interval (1.46 Hz intervals for the wider beta waveband). Alpha was the only waveband that showed any marked (non-significant) increase in power.

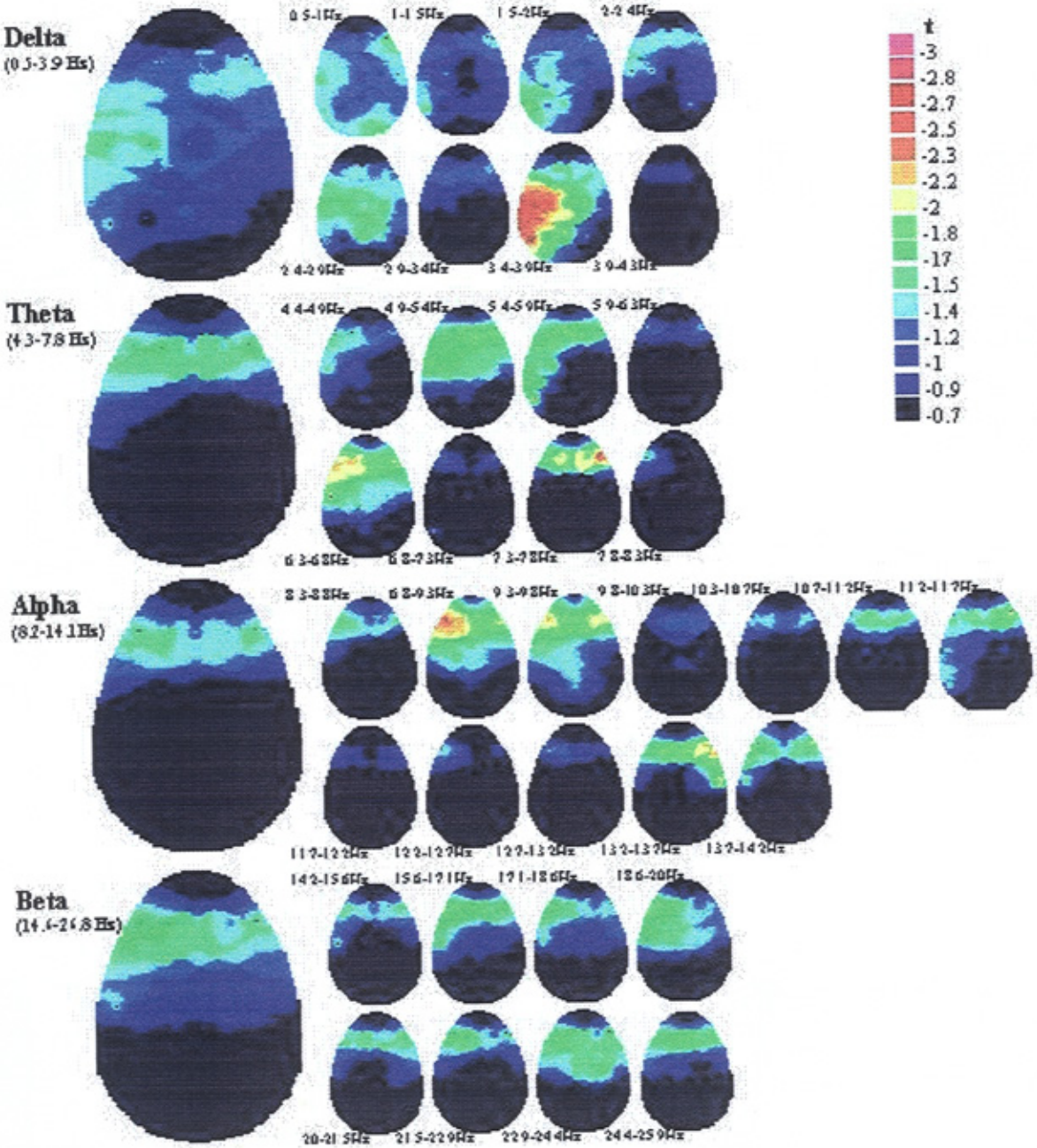


*Figure 8.1. Descriptive topographic maps (paired t tests) of each waveband, showing smoothed reductions in EEG power, in comparison to placebo, following 360 mg of Ginkgo biloba. Larger maps represent power averaged across the entire waveband (delta, theta, alpha and beta), and the smaller maps represent comparisons at the individual 0.487 Hz (1.46 Hz for the wider beta waveband) intervals that make up the larger waveband.*

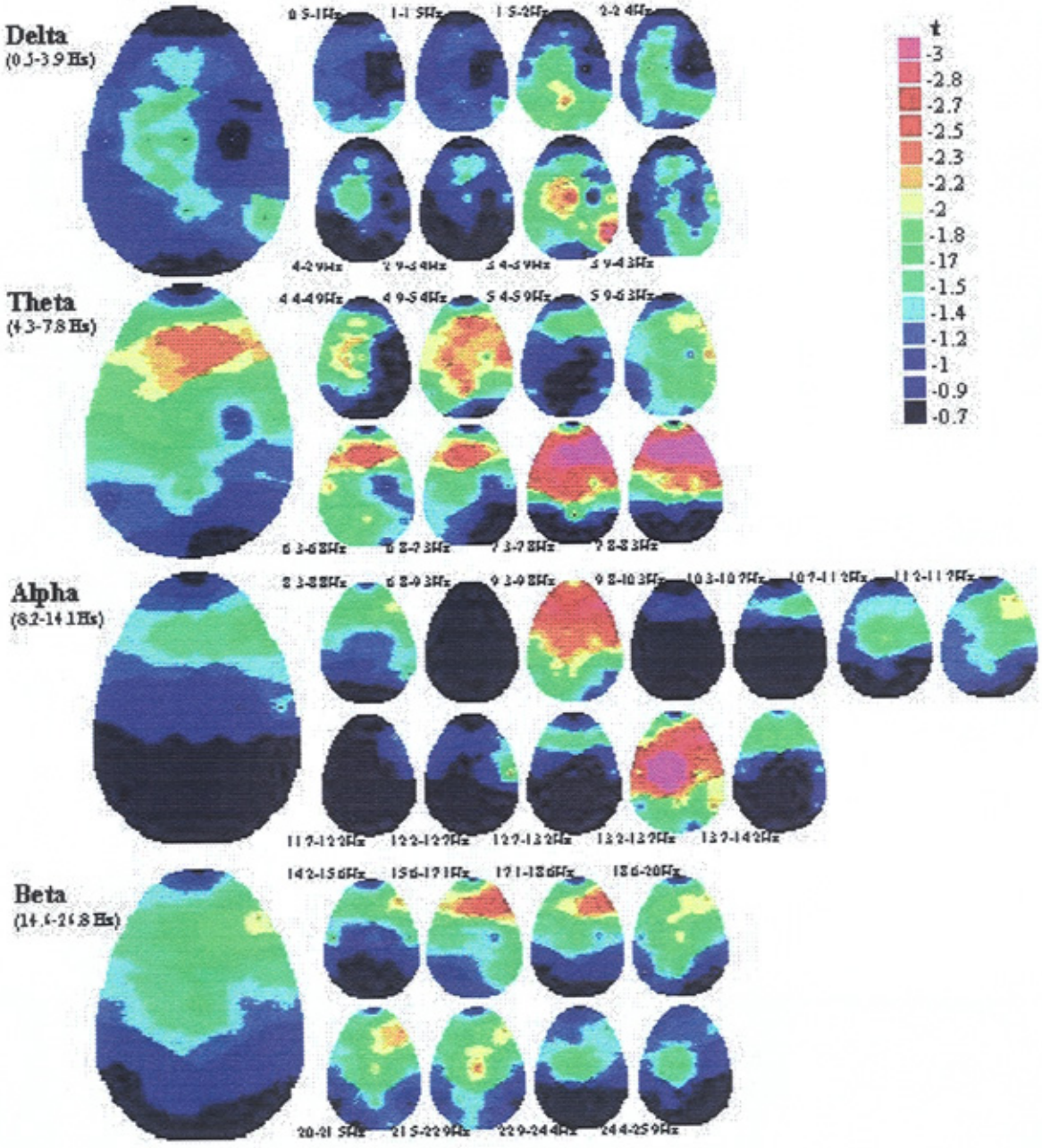


*Figure 8.2. Descriptive topographic maps (paired t tests) of each waveband, showing smoothed reductions in EEG power, in comparison to placebo, following 200 mg of Panax ginseng. Larger maps represent power averaged across the entire waveband (delta, theta, alpha and beta), and the smaller maps represent comparisons at the individual 0.487 Hz (1.46 Hz for the wider Beta waveband) intervals that make up the larger waveband.*

### Cognitive Measures

There were no significant differences on either the cognitive or mood measures. Mean scores for the three cognitive factors, and the measures derived from the Bond Lader mood scales are presented in Table 8.2.

| *Measure* | placebo Mean | s.e. | ginkgo Mean | s.e. | ginseng Mean | s.e. |
|---|---|---|---|---|---|---|
| **Secondary Memory** (% x 3) | **176.44** | *9.41* | **182.22** | *9.93* | **178.67** | *12.67* |
| **Speed Of Attention** (ms X 3) | **1029.1** | *27.7* | **1042.3** | *24.3* | **1046.6** | *31.42* |
| **Accuracy of Attention** (%) | **96.51** | *0.53* | **96.60** | *0.55* | **96.18** | *1.13* |
| **Alert** (mm) | **52.04** | *3.85* | **54.77** | *3.67* | **56.03** | *3.99* |
| **Content** (mm) | **68.37** | *3.85* | **68.63** | *3.57* | **65.37** | *3.66* |
| **Calm** (mm) | **68.50** | *4.10* | **64.93** | *3.85* | **59.43** | *4.94* |

*Table 8.2. Mean scores (and standard errors) for the cognitive factors 'Secondary Memory', 'Speed of Attention', and 'Accuracy of Attention', and the Bond-Lader visual analogue scale mood outcomes, 'alert', 'calm', and 'content', following placebo, Ginkgo biloba, and Panax ginseng.*

### Correlation of Cognitive Performance and EEG

Correlation (Pearson's) of the change in EEG measures (P300 latency and amplitude, and 'eyes closed' power) between conditions at each electrode (treatment minus placebo), and the change in performance on the three cognitive factors (treatment minus placebo), revealed a number of significant relationships. Due to the large number of correlations involved (17 per EEG/factor comparison), and the intrinsic possibility of individual significant correlations occurring by chance, only those significant correlations that fall into coherent patterns are presented. For those measures that evinced interpretable patterns, r scores and probabilities at each electrode are presented in Figure 8.3. Due to the exploratory nature of the analysis the correlation results

are reported as descriptive topographic arrays, with no correction having been made for multiple comparisons.

***Ginseng***

Despite the absence of any significant modulation of cognitive performance across the cohort of participants there were still a number of significant (uncorrected) correlations that can be interpreted as reflecting a relationship between treatment related localised changes in individuals' EEG and the change in their cognitive performance. Change in P300 amplitude (in comparison to placebo) correlated positively with both change in 'Secondary Memory' performance (i.e. improved performance) in electrodes within the central, left temporal, and left frontal areas, and slowing on the 'Speed of Attention' factor across the frontal electrodes. The faster P300 latency evinced in this condition also correlated with decreased 'Accuracy of Attention', largely across the central electrodes. Change in 'Accuracy of Attention' was also positively correlated with the decrease in power of the theta waveband across frontal electrodes.

***Ginkgo***

Change (in comparison to placebo) in the power of the alpha waveband was significantly correlated with change in 'Speed of Attention' at a number of electrodes. However these correlations were bi-directional, with change in power across the occipital electrodes, at which alpha power was significantly increased overall, negatively correlating with speed on the attention tasks (i.e. the increase in power was associated with improved performance in terms of reduction in milliseconds). In contrast the power of the alpha waveband at parietal/central electrodes was positively correlated with speed on the attention tasks.

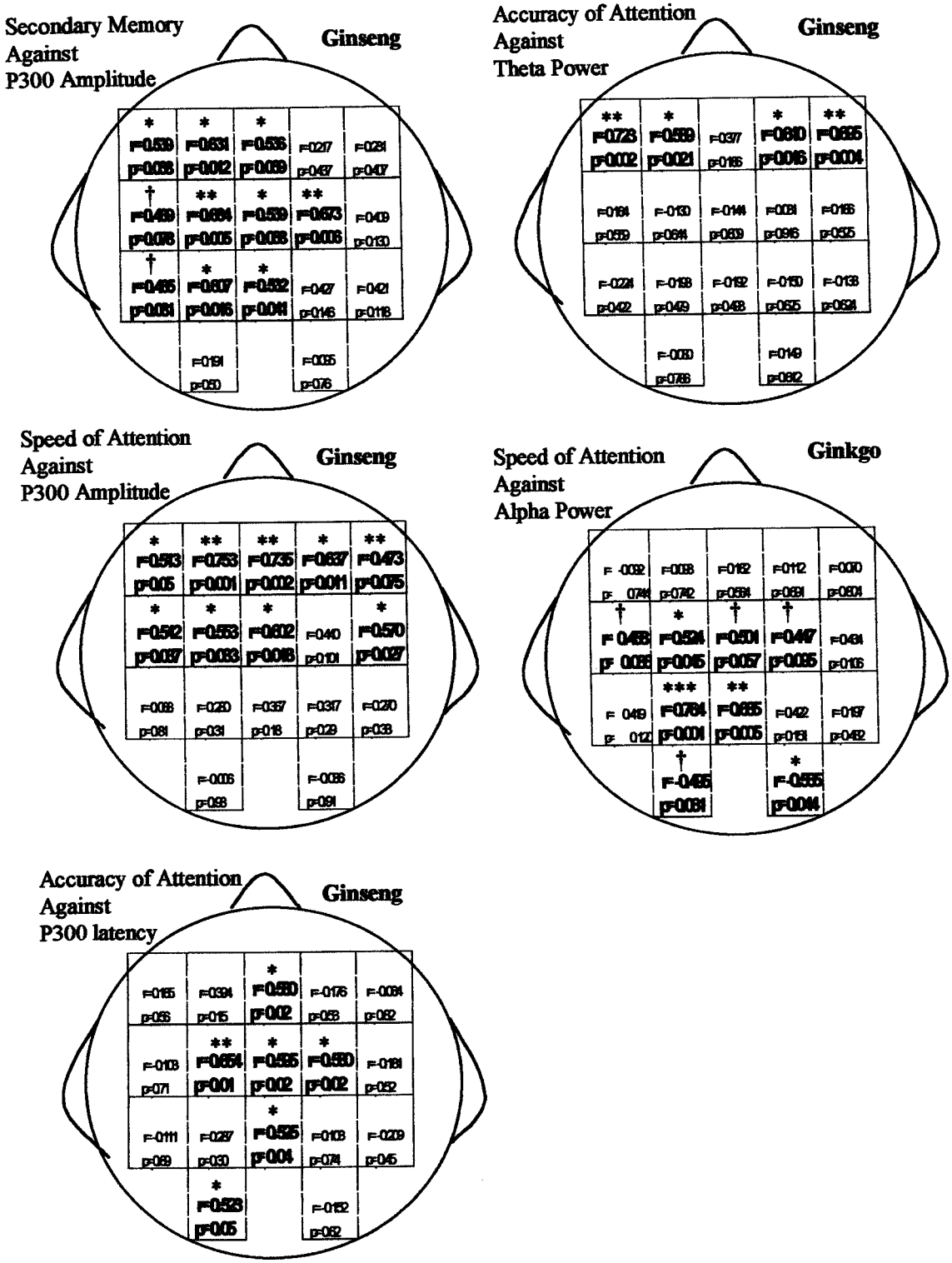


*Figure 8.3. Descriptive arrays of Pearson's product moment correlations (r) and associated probabilities at each electrode for correlations between the change following the relevant treatment (in comparison to placebo) in EEG measures and the change in Cognitive factor scores († = P<0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001).*

## 8.4. Discussion

The results of this study show that acute doses of *Ginkgo biloba* and *Panax ginseng* are capable of modulating cerebral bioelectrical activity as measured by EEG. In both cases acute oral ingestion of the treatment was associated with significant decreases in both theta and beta wavebands, predominantly in frontal scalp areas. Ginseng also resulted in reduced frontal alpha activity. In contrast to our expectation, based on previous demonstrations of faster and slower speeds for ginkgo and ginseng respectively in the performance of tasks assessing attention (Chapter 2, 3), only ginseng was associated with modulation of evoked potentials, with decreased latency for the P300 wave. Neither treatment altered mood or cognitive performance on this occasion.

Previous research into the EEG effects of *Ginkgo biloba* in elderly, pathological populations has suggested that the extract's most notable effect is one of reducing the proportion of resting, 'eyes closed' theta activity in comparison to alpha activity. Following the ingestion of ginkgo a reduction in theta activity has been demonstrated in cohorts suffering from Alzheimer's disease and vascular dementia (Hofferberth, 1994), and cerebral insufficiency (Hofferberth, 1995). Similarly, decreases in theta activity with concomitant increases in alpha activity have been shown in patients suffering cerebral insufficiency (Pidoux *et al,* 1983; Schulz *et al,* 1991), and in sub groups of 'responders' within cohorts suffering from dementia (Itil *et al,* 1998) and age related mental deterioration (Geβner *et al,* 1985). These latter studies present less than compelling evidence of overall group differences in the EEG as a result of ingestion of ginkgo. Nevertheless, it is of interest to note that in the current study, despite being undertaken in a healthy younger cohort, not only was resting, 'eyes closed' theta power significantly reduced across frontal electrodes, but also the relative proportion of theta activity in comparison to alpha activity across the whole scalp was reduced following ginkgo by 3.8% (data not shown). Little support was found, however, for the suggestion that the effects of ginkgo in healthy cohorts is typified by increased alpha activity (Itil *et al,* 1996; Luthinger *et al,* 1995). Indeed in

this instance the pattern of alpha modulation was for decreased frontal alpha, and increased occipital alpha, both of which narrowly failed to reach significance (following log-transformation of the data). It is also noteworthy that whilst Itil *et al* (1996) showed a clear increase in alpha power in a cohort of 18-65 year olds, one of two papers which have reported topographic results following ginkgo in young cohorts (<40 years) showed increased frontal alpha 1 (8-9.5 Hz) absolute power, with a concomitant trend towards reduced alpha 2 (10-12.5 Hz) absolute power in the same region (Luthinger *et al*, 1995). The other paper (Kunkel, 1993) reported significant alpha modulation restricted to one significant increase, and one significant decrease in temporal absolute alpha power following different doses of Ginkgo (80 and 160 mg EGb761 respectively). It is still tempting to speculate that the bi-directional pattern of topographic alpha results seen in the current study may reflect a beneficial modulation of alpha activity overall.

The expected modulation by *Ginkgo biloba* of the event related potentials, for which there is some evidence in both sufferers from age associated memory impairment (Semlitsch *et al*, 1995) and healthy young volunteers (Luthinger *et al*, 1995), was not evident. Reference to the latter of these papers suggests that the time profile of both positive and negative changes in both CNV and the P300 wave following ginkgo are far from straightforward, and a single 4 hour post-dose 'snapshot' of EEG, as in the current study, may not be a suitable approach to investigating such effects.

The present study represents the first EEG investigation of a ginseng extract's ability to modulate bio-electrical cerebral activity. In the absence of any benchmark results with which to make a comparison, it seems most parsimonious to suggest that the profile of results established here following *Panax ginseng* has a number of similarities to those evinced by *Ginkgo biloba*. No direct statistical comparison was made between the two active treatments. so it is only possible to suggest, certainly on the basis of the topographic probability maps and additional statistical evidence, that the EEG effects of this dose of *Panax ginseng* (200 mg G115) are demonstrably stronger than those for the dose of *Ginkgo biloba* utilised (360 mg GK501). The

effects of ginkgo were restricted to significant treatment/scalp area interactions, with significant frontal power reductions for theta and beta bands. Ginseng elicited the same topographic pattern of interactions, but with the addition of significantly reduced frontal alpha and significant *post-hoc* treatment effects for mean power across the whole head on both the theta and beta bands. The relative proportion of theta activity in comparison to alpha activity across the whole scalp was also reduced by ginseng by 7.9%, in comparison to 3.8% for ginkgo. It is also notable that ginseng also evinced the same pattern of alpha modulation, with frontal reductions coupled with a greater numerical increase in alpha power in the occipital region.

This raises the possibility that the effects seen here for the two extracts are dependent on the same mechanisms. It has been suggested that the beneficial effect of ginkgo on EEG is related to an upregulation of cholinergic function (Itil *et al,* 1996; Luthinger *et al,* 1995). Both ginkgo (Chopin and Briley, 1992; Huguet *et al,* 1994; Kristoikova and Klaschka, 1997; Taylor, 1986), and ginseng (Benishin *et al,* 1991; Benishin 1992; Hsieh *et al,* 2000; Jin *et al,* 1999; Lewis *et al,* 1999) are reported to modulate aspects of cholinergic functioning. It is possible therefore that they have an effect on EEG activity through a number of cholinergic subcortical structures that are thought to underlie the rhythmicity and synchronisation of cerebral bioelectrical activity (e.g. Fisch, 1999; Steriade *et al,* 1993).

However, ginkgo and ginseng also share a number of other common physiological effects, including: anti-oxidant properties (Mantle *et al,* 2000; Siddique *et al,* 2000); an effect on platelet aggregation (Braquet and Hosford, 1991; Jung *et al,* 1998; Shi *et al,* 1990; Smith *et al,* 1996;), and modulation of other haemorrheological parameters (Gillis, 1997; Jung *et al,* 1990; Kriegelstein *et al,* 1986). It is possible that these or other properties allow both extracts to promote increased delivery of metabolic substrates to the brain *per se.*, although, in this respect the direct evidence of such an effect is stronger at this time for *Ginkgo biloba* (see Chapter 5).

It is also particularly interesting to note that the fractionation of the topographic maps into 0.487 Hz intervals (1.46 Hz for Beta) suggests that the modulation of activity by both extracts is restricted to specific frequencies within the conventional wavebands, and that the pattern has

similarities for both extracts. As an example, in the delta band both extracts peak modulation of activity comes in the 3.4-3.9 Hz frequency. In the theta band both have one of their strongest decreases in power at a frequency of 7.3-7.8 Hz, and in the alpha band both have specific decreases in the 9.3-9.8Hz and 13.2-13.7Hz intervals. Whilst the relevance of these 'hotspots' of reduced activity is as yet unclear, it is possible to speculate that this decrease in specific frequency bands within summated cortical activity may reflect common regulation of the amplitude of frequency specific subcortical mechanisms.

One way in which the effect of ginseng does differ markedly from that of ginkgo in the current study is the demonstration of reduced P300 latency. Our original hypothesis, derived from the previous observation of slowed performance on the 'Speed of Attention' cognitive factor following a 200 mg dose of ginseng (Chapter 3), was that latency would be increased. In the current study neither extract engendered significant differences on cognitive performance. This absence can be most parsimoniously ascribed to methodological limitations, with a lack of a baseline cognitive measurement, a reduction in the cohort size, and therefore the statistical power to detect relatively subtle cognitive differences, and limitations in the practical set up of the cognitive testing within the somewhat lengthy and uncomfortable EEG study setting. Despite this, improved P300 latency following ginseng was still somewhat unexpected, and ran directly counter to our original hypothesis.

Interestingly, despite an absence of cognitive modulation, the exploratory correlation analysis demonstrated several topographical relationships between the differences in cognitive performance and EEG that were evinced following each of the treatments. In the case of ginkgo there was only one interpretable pattern of correlations, with both increases at occipital electrodes and decreases at central electrodes in alpha power being correlated with improved 'Speed of Attention'. It is tempting to suggest that this might indicate that the bi-directional nature of the alpha modulation following ginkgo (decreased frontal alpha, and increased occipital alpha) represents an overall beneficial modulation. Ingestion of ginseng was associated with a number of localised patterns of correlations, with both improved secondary

memory performance and decreased speed on attentional tasks being associated with increased P300 amplitude. Decreased theta power and decreased P300 latency were also associated with decreased accuracy on the attention tasks (frontally and centrally respectively). It is possible to speculate that, even in the absence of cognitive modulation, these correlations may in some way reflect individual differences in cerebro-electrical and cognitive responses to the ingestion of ginseng. Whilst this interpretation is necessarily tentative, it also has to be acknowledged that the pattern of relationships may simply reflect the cerebro-electrical correlates of chance individual fluctuations in cognitive performance between testing sessions. However, if this is the case it seems curious that such effects should largely be restricted to the ginseng condition. Several of the above observations and tentative interpretations are necessarily speculative. However, the current study has served to confirm that acute doses of *Ginkgo biloba* exert effects on cerebral bio-electrical activity in healthy, young volunteers. Moreover, it represents the first investigation demonstrating EEG effects following *Panax ginseng,* or indeed any *Panax* species. In this respect the demonstration of a comparatively stronger EEG effect associated with ginseng, coupled with demonstrations of memory improvements (Chapters 3 and 7), suggest that the efficacy of ginseng as a cognition enhancer might usefully benefit from a similar level of adequately controlled research as that which has been directed towards *Ginkgo biloba.*

# CHAPTER 9. COGNITIVE EFFECTS OF ACUTE ADMINISTRATION OF *SALVIA LAVANDULAEFOLIA* (SPANISH SAGE)

## 9.1. Introduction

Plants of the *Salvia* genus have a pan-cultural history of usage, with traditional medicinal applications in, amongst others, ancient Greek, Roman (Ryman, 1991), Ayurvedic (MacIntyre, 1996), indigenous American Indian (Moerman, 1986) and traditional Chinese medical systems (Hsu *et al,* 1986; Perry *et al,* 1998).

*Salvia officinalis* was in common usage throughout Europe by medieval times, and features in British herbal apothecaries from the 16$^{th}$ century onwards (Crellin and Philpott, 1990). Its suggested uses included those as a general treatment to enhance 'head and brain' functioning, improve the memory, quicken the senses, and delay age associated cognitive decline (Perry *et al,* 1999). The many contemporary indications for *S. officinalis* include the alleviation of poor memory, mental confusion, depression, vertigo, as an anti-inflammatory, and use as a treatment for the symptoms of the menopause (Bartram, 1998; British Herbal Pharmacopoea, 1983).

*Salvia lavandulaefolia* (Spanish Sage) has a similar composition to *S. officinalis*, with the exception that it lacks a high concentration of thujone, which is toxic in large doses. It has therefore been suggested that *S. lavandulaefolia* may provide an equally efficacious, but more theoretically suitable, treatment (Mantle *et al,* 2000). In terms of the whole herb, both *Salvia* species contain about 1.0 – 2.8 % volatile oil (Leung and Foster, 1996), and it has been suggested that the monoterpenoid constituents (α-Pinene, β-Pinene, 1,8-Cineole, Thujone, Camphor, Geraniol), comprise some of the active components of the whole herb (Perry *et al,* 2001).

Both *Salvia officinalis* and *lavandulaefolia* have been reported to have a number of *in vitro* properties. These include demonstrations for both of concentration dependent inhibition of acetylcholinesterase (AChE) in human brain homogenates *in vitro* as a consequence of the application of both the essential oil, and alcoholic extracts of both fresh and dried leaves (Perry

*et al,* 1996). In a similar vein, Perry *et al* (2000) demonstrated dose dependent inhibition of erythrocyte AChE by *S. lavandulaefolia* essential oil, but found that no single constituent was particularly potent, suggesting a synergistic relationship. This *in vitro* anti-cholinesterase activity of the essential oil of *S. lavandulaefolia* has also been confirmed *ex vivo,* with the demonstration of a similar effect as physostigmine on the contractile response of the isolated guinea pig ileum (Perry *et al,* 2001), and *in vivo* with inhibition of AChE in selected brain areas following oral administration of *S. lavandulaefolia* to aged rats (Perry *et al,* 2002).

It has also been reported that *Salvia officinalis* leaf had 'appreciable' levels of anti-oxidant activity, in comparison to recognised antioxidants such as *Ginkgo biloba* and *Panax ginseng* (Mantle *et al,* 2000a). Anti-oxidant properties of the essential oil of *S. Lavandulaefolia* (Perry *et al,* 2001), and a number of single constituents common to both *S. officinalis* and *S, lavandulaefolia* have also been reported (e.g. Djarmati *et al,* 1992; Dorman *et al,* 1995; Lamaison *et al,* 1993; Perry *et al,* 2001).

*In vitro* research also lends support to the anti-inflammatory and oestrogenic properties that have been historically attributed to *Salvia* species (Bartram, 1995), with demonstrations of anti-inflammatory actions by an ethanol extract of *S. Lavandulaefolia* and several of its constituents, and human oestrogen receptor binding activity by an essential oil, and its monoterpenoid component *geraniol* (Perry *et al,* 2001).

While no study has assessed the effects of sage in humans, it has been suggested, on the basis of the above mechanisms, that *S. lavandulaefolia* may provide a novel treatment for Alzheimer's disease (Mantle *et al,* 2000b; Perry *et al,* 1996; Perry *et al,* 1998). In particular the plant's acetylcholinesterase inhibitory properties, which may engender a consequent increase in synaptically available acetylcholine, could potentially serve to ameliorate the cognitive disturbances associated with cholinergic neuron and receptor loss and dysregulation, without the attendant side effects of currently available treatments (Perry *et al,* 1998). Added anti-inflammatory and antioxidant properties may also convey additional benefits, while interaction with oestrogen receptors raises the possibility of further potentially beneficial effects, including

increased cerebral blood flow, anti-inflammatory actions, and neuroprotective and neurotrophic effects in brain tissue (Shepherd, 2001). It is particularly germane to the current study that these demonstrated properties should also, theoretically, be capable of modulating cognitive performance in healthy populations.

The current study will therefore represent the first investigation of the effects of sage in humans. The dose and time-dependent cognitive and mood effects of ingestion of 2 single doses of the essential oil of *Salvia lavandulaefolia* (and a placebo) will be assessed in healthy young volunteers using the CDR computerised assessment battery, serial subtraction tasks, and the Bond-Lader mood scales.

## 9.2. Materials and Methods

**Essential oil and *in vitro* analysis.**

*S. lavandulaefolia* essential oil was obtained from Baldwins & Co. (London). The main constituents of the oil were identified using gas chromatography utilising the methodology described in Perry *et al* (2001; 2002).

The human erythrocyte acetylcholinesterase inhibitory properties of the essential oil were investigated using the methodology described in Perry *et al* (2000).

The above analyses were undertaken at the MRC Centre for Development in Clinical Brain Ageing, Newcastle General Hospital.

**Participants**

16 female and 8 male undergraduate volunteers (mean age 23.2 years, range 18-37) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Habitual smokers were excluded from the study. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

**Cognitive Measures**

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive measures were as described in detail previously in Chapter 2 (section 2.2. pages 87-93).

**Serial subtraction tasks**

Modified computerised versions of the Serial Threes and Serial Sevens tasks, as described in detail in Chapter 5 (pages 141-142) were employed.

**Subjective mood measure**

Bond-Lader Visual Analogue Scales (Bond and Lader 1974) were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

**Treatments**

The essential oil was encapsulated with sunflower oil. On each study day participants received three visually identical capsules containing either sunflower oil (placebo), or sunflower oil plus 25 µl of essential oil..

Depending on the condition to which the participant was allocated on that particular day the combination of capsules corresponded to a dose of either 0 (placebo), 25 µl, or 50 µl of *S. lavandulaefolia* essential oil (these essential oil quantities correspond to approximately 1.25 g and 2.5 g of dried leaf respectively).

**Procedure**

Each participant was required to attend a total of four study days that were conducted seven days apart to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the three active days of the study.

The first day was identical to the following three, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment on visits 2 to 4. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader Visual Analogue Scales, the CDR test battery, and finally the Serial 3s and Serial 7s computerised subtraction tasks.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, the individual task outcome measures, Serial 3s and Serial 7s scores, and the three mood outcomes derived from the Bond-Lader visual analogue scales, were analysed as 'change from baseline' using the Minitab statistical package. The initial analysis was made using a two factor (condition x session) Analysis of Variance with repeated measures on both factors. Following the recommendations of Keppel (1991), the omnibus F test was eschewed in favour of planned comparisons, which were made between the

placebo and each of the two active treatment conditions (25 µl and 50 µl of *S. lavandulaefolia* essential oil) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

## 9.3. Results

### AChE Inhibition and Gas Chromatography

The *S. lavandulaefolia* essential oil inhibited AChE in a dose dependent manner, generating an $IC_{50}$ value of 0.08 mg/ml. This compares favourably with previous such analyses for the same source of essential oil (Perry *et al,* 2000).

The main monoterpenoid constituents of the essential oil, as identified by gas chromatography, are shown in Table 9.1.

| Monoterpenoid constituent | Relative % of essential oil |
|---|---|
| α- Pinene | 6.5 |
| Camphene | 6.3 |
| β- Pinene | 5.4 |
| Myrlene | 1.9 |
| Limonene | 1.2 |
| 1,8- Cineole | 25.8 |
| Camphor | 24.4 |
| Caryophyllene | 1.2 |
| Terpinen-4-OL | 2.0 |
| Borneol | 3.3 |
| α- Terpineol | 2.8 |

*Table 9.1.main constituents of the S. lavandulaefolia essential oil as determined by gas chromatography. Identities of individual compounds were determined from the retention times.*

### Baseline cognitive and mood scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all three conditions (placebo, 25 µl, and 50 µl) for each of the primary outcome measures ('Quality of Memory' measure, 5 cognitive factors, mood scale scores, serial subtraction scores) were subjected to a one-way, repeated-measures, Analysis of Variance. There was a single significant difference in baseline performance [$F(2,46) = 3.5$, $p = 0.039$], with *post-hoc* comparisons (Dunnett's) showing that while in the 50 µl condition participants scored less errors ($p < 0.05$) on the Serial 3s task than while in the placebo condition.

**Individual task outcome measures**

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are presented in Table 9.1. Significant results on individual task outcomes are presented in relationship to the overall measure to which they contribute below (memory task results are presented with either. 'Secondary' or 'Working' memory).

**Cognitive factor outcome measures**

Mean raw and change from baseline cognitive factor outcome measure scores for each condition across each session are displayed in the table and graphs of Figure 9.1.

***Quality of Memory measure***

Planned comparisons revealed a significant improvement in the accuracy of memory task performance, in comparison to placebo, for the 25 µl dose of sage at 1 hour post-dose [$t(138) = 2.85$, $p = 0.005$]. There was also a trend towards improved performance for the same dose at 4 hours post-dose [$t(171) = 1.71$, $p = 0.09$].

***Secondary Memory Factor***

The 25 µl dose of sage was associated with a significant improvement on the 'Secondary Memory' factor at 1 hour post-dose [$t(138) = 2.66$, $p = 0.009$]. There was also a strong trend

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Immediate Word Recall (% accuracy) | placebo | 52.36 3.45 | -9.86 3.31 | -5.28 3.37 | -10.83 4.19 | -4.72 2.05 |
| | 25 µl | 49.44 3.40 | -3.06 3.38 | -8.06 2.78 | -5.83 3.30 | -9.58 3.32 |
| | 50 µl | 48.47 2.87 | -2.08 3.43* | -1.67 3.09 | -3.89 2.40* | -5.14 2.76 |
| Simple Reaction time (msecs) | placebo | 262.05 6.58 | 6.79 5.48 | 12.29 5.17 | 13.83 7.84 | 13.93 4.66 |
| | 25 µl | 262.82 6.63 | 3.67 5.50 | 18.17 16.36 | 6.17 6.92 | 8.72 6.05 |
| | 50 µl | 293.98 29.45 | -18.42 5.81 | -15.17 29.2 | -11.10 27.43 | -22.30 30. |
| Digit Vigilance Accuracy (%) | placebo | 97.78 0.77 | -1.94 1.47 | -1.67 1.28 | -2.22 1.69 | -0.56 1.13 |
| | 25 µl | 97.78 0.77 | -1.11 1.47 | -2.50 1.49 | -1.67 1.35 | 0.28 0.94 |
| | 50 µl | 97.50 0.88 | -0.56 1.43 | -1.39 1.13 | -2.50 1.54 | -2.22 1.95 |
| Digit Vigilance False alarms (number) | placebo | 0.33 0.12 | 0.21 0.18 | 0.54 0.28 | 0.17 0.19 | 0.08 0.18 |
| | 25 µl | 0.58 0.16 | 0.00 0.17 | -0.17 0.22 | -0.13 0.20 | -0.04 0.24 |
| | 50 µl | 0.25 0.11 | 0.17 0.20 | 0.25 0.18 | 0.08 0.13 | 0.42 0.20 |
| Digit Vigilance Reaction time (msecs) | placebo | 387.28 7.91 | 1.52 5.95 | 7.27 5.68 | 16.55 8.96 | 13.82 7.08 |
| | 25 µl | 373.08 7.78 | 2.10 5.95 | 15.97 7.16 | 25.64 8.10 | 15.80 7.15 |
| | 50 µl | 380.16 7.98 | 14.95 6.14 | 20.36 8.63 | 24.65 8.53 | 20.28 6.96 |
| Choice reaction time accuracy (%) | placebo | 94.00 0.73 | 0.50 0.70 | -1.50 0.69 | -1.42 0.97 | -1.83 0.92 |
| | 25 µl | 94.75 0.76 | -1.42 0.69 | -2.33 0.80 | -1.33 1.18 | -2.17 0.81 |
| | 50 µl | 94.50 0.92 | -0.42 0.77 | -1.00 1.06 | -1.67 0.76 | -1.75 0.96 |
| Choice reactionTime (msecs) | placebo | 405.50 13.48 | 7.25 8.04 | 11.53 9.22 | 27.06 10.44 | 5.91 9.41 |
| | 25 µl | 423.82 15.89 | -5.93 8.01 | -4.67 7.77 | -10.57 10.10*** | -7.42 7.15 |
| | 50 µl | 414.16 15.44 | 0.08 7.98 | -2.02 11.41 | 17.93 8.77 | -1.75 8.87 |
| Spatial Memory (%>chance) | placebo | 92.24 1.61 | -3.39 2.13 | -3.13 2.02 | -6.04 2.04 | -11.15 3.41 |
| | 25 µl | 93.33 1.37 | -0.99 1.76 | -9.22 4.52 | -9.01 3.64 | -10.21 5.30 |
| | 50 µl | 91.72 1.73 | -6.09 1.76 | -4.27 3.07 | -12.60 5.91 | -6.67 4.54 |
| Spatial memory Reaction time (msecs) | placebo | 566.31 19.61 | -26.51 12.30 | 14.76 16.19 | -10.31 13.41 | 25.88 29.45 |
| | 25 µl | 556.76 26.52 | 1.60 11.87 | -20.12 23.71 | -17.07 21.27 | -27.27 19.81 |
| | 50 µl | 607.64 44.66 | -39.62 11.95 | -70.78 38.52**** | -59.92 40.02* | -64.63 46.65***** |
| NumericWorking Memory (%>chance) | placebo | 88.61 1.60 | -6.11 1.81 | -3.33 1.63 | -6.39 2.83 | -5.46 2.97 |
| | 25 µl | 89.08 1.58 | -1.67 1.72* | -3.43 1.80 | -3.06 2.00 | -7.22 3.10 |
| | 50 µl | 89.45 1.38 | -3.06 1.73 | -3.52 1.70 | -5.56 1.73 | -5.28 2.25 |
| Numeric Working Memory Reaction Time (msecs) | placebo | 532.71 17.91 | -18.98 10.68 | -12.02 9.17 | -8.25 14.88 | -25.44 12.74 |
| | 25 µl | 556.00 24.68 | -16.67 9.47 | -34.50 11.91* | -29.01 13.47 | -27.87 16.18 |
| | 50 µl | 564.38 25.27 | -17.37 9.35 | -26.79 11.93 | -19.85 11.72 | -29.43 12.11 |
| Delayed Word Recall (% accuracy) | placebo | 37.36 3.39 | -12.08 2.79 | -8.47 2.84 | -14.86 3.63 | -11.25 2.34 |
| | 25 µl | 34.72 3.66 | -6.39 2.71 | -10.28 3.23 | -11.39 3.49 | -12.92 2.73 |
| | 50 µl | 35.83 3.13 | -8.47 2.71 | -12.64 2.84 | -8.75 3.65 | -12.22 3.67 |
| Word Recognition (%>chance) | placebo | 69.44 2.95 | -8.89 3.88 | -15.47 3.67 | -17.13 4.06 | -13.46 4.41 |
| | 25 µl | 64.44 4.20 | -7.22 3.88 | -10.78 4.03 | -8.05 4.38* | -15.00 5.08 |
| | 50 µl | 63.06 3.45 | -7.22 3.99 | -10.28 4.47 | -8.89 3.60* | -10.56 4.11 |
| Word Recognition Reaction time (msecs) | placebo | 638.56 24.50 | -29.44 19.12 | 23.98 25.10 | 34.30 38.83 | -27.34 19.78 |
| | 25 µl | 635.94 23.80 | 26.13 18.86 | 2.01 17.20 | 18.02 15.77 | -10.24 17.23 |
| | 50 µl | 645.56 23.30 | -13.26 18.75 | 36.28 26.71 | -4.87 12.01 | -14.12 16.53 |
| Picture Recognition (%>chance) | placebo | 67.92 3.36 | -11.67 4.40 | -12.71 4.54 | -5.00 4.17 | -5.83 3.88 |
| | 25 µl | 64.58 3.11 | 0.42 4.57** | -2.71 3.57* | -1.67 4.07 | -1.88 3.38 |
| | 50 µl | 67.71 3.70 | -5.83 4.68 | -6.46 3.59 | -10.21 4.05 | -3.96 3.72 |
| Picture recognit'n Reaction time (msecs) | placebo | 712.61 21.17 | -2.92 14.01 | 20.42 18.83 | 34.66 25.03 | 8.31 23.37 |
| | 25 µl | 744.41 25.54 | -8.29 14.02 | -20.09 22.82* | -16.33 20.72** | -31.64 19.82* |
| | 50 µl | 757.66 23.91 | 29.26 13.58 | 33.58 21.99 | -21.42 21.68*** | -30.53 23.61* |

*Table 9.2. Effects of Salvia lavandulaefolia essential oil on individual task outcome measures from the CDR battery. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.005$, ****, $p < 0.001$; *****, $p < 0.0005$ compared to placebo).*

towards improved performance for the same dose at 4 hours post-dose [$t(138) = 1.96$, $p = 0.052$]. Reference to the single tasks showed that 50 µl evinced a greater improvement than placebo on the immediate word recall task at 1 hour [$t(138) = 2.24$, $p = 0.026$] and 4 hours [t(138) = 2, p = 0.047 ]. There was a strong trend towards improvement for the 25 µl dose at 1 hour post-dose [t(138) = 1.96, p = 0.052]. Word recognition task performance was also improved for both doses at the 4 hour testing session ( 25 µl [t(138)=2.26, p=0.025] and 50 µl. [t(138) = 2.05; p = 0.04]), whilst delayed picture recognition task performance was improved at 1 and 2.5 hours for the 25µl dose ([t(138) = 2.79, p = 0.006] and [t(138) = 2.3, p = 0.022].

***Working Memory Factor***

There were no significant differences on the 'Working Memory' factor. There was, however, a single significant improvement on the numeric working memory task at 1 hour post-dose for 25 µl of sage oil [t(138) = 2.2, p = 0.029]. Given the nature of the statistical analysis it seems unwise to over-interpret such single significant comparisons.

***Speed of Memory factor***

Performance on the 'Speed of Memory' factor was improved for both doses of essential oil, with participants performing faster, relative to placebo, in the 25 µl condition at 2.5 hours [$t(138) = 2.34$, $p = 0.021$] with a trend towards the same at 4 hours post-dose [$t(138) = 1.85$, $p = 0.066$], and following 50 µl at 4 [$t(138) = 3.05$, $p = 0.003$] and 6 hours post-dose [$t(138) = 2.35$, $p = 0.02$]. Reference to the individual tasks suggests that both doses of essential oil were associated with a greater increase in speed than placebo on the delayed picture recognition task, with improvements associated with 25 µl at 2.5 hours [$t(138) = 2.08$, $p = 0.039$], and with both 25 µl and 50 µl at 4 hours ([$t(138) = 2.61$, $p = 0.01$] and [$t(138) = 2.88$, $p = 0.005$]

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Quality of Memory (%X6) | placebo | 402.10 *9.55* | -51.16 *11.12* | -45.88 *6.81* | -58.87 *12.20* | -49.65 *10.92* |
| | 25 µl | 391.44 *12.10* | -16.68 *10.82* | -47.52 *9.31* | -38.18 *12.61* | -59.03 *12.75* |
| | 50 µl | 392.90 *10.68* | -34.98 *11.12* | -41.61 *10.32* | -51.84 *13.14* | -43.82 *11.79* |
| Secondary Memory (%X4) | placebo | 221.25 *10.13* | -41.67 *10.10* | -39.43 *6.80* | -46.44 *12.22* | -33.04 *7.80* |
| | 25 µl | 209.03 *11.71* | -14.03 *10.07* | -34.88 *9.08* | -26.11 *10.14* | -41.60 *9.52* |
| | 50 µl | 211.74 *10.28* | -25.83 *10.47* | -33.82 *9.68* | -33.68 *10.26* | -31.88 *9.96* |
| Working Memory (%X2) | placebo | 180.85 *2.68* | -9.50 *3.61* | -6.46 *2.44* | -12.43 *2.84* | -16.61 *5.25* |
| | 25 µl | 182.41 *2.46* | -2.66 *3.12* | -12.65 *4.87* | -12.07 *4.54* | -17.43 *6.58* |
| | 50 µl | 181.17 *2.33* | -9.15 *3.14* | -7.79 *4.15* | -18.16 *6.45* | -11.94 *5.70* |
| Speed of Memory (summed msecs) | placebo | 2450.2 *69.14* | -77.84 *34.09* | 47.15 *51.38* | 50.40 *67.73* | -18.59 *64.29* |
| | 25 µl | 2493.1 *87.58* | 2.77 *30.98* | -72.70 *49.16* | -44.38 *45.62* | -97.02 *51.71* |
| | 50 µl | 2575.2 *90.14* | -40.98 *30.76* | -27.71 *69.06* | -106.1 *60.26* | -138.7 *81.83* |
| Quality of Attention (%) | placebo | 90.67 *0.52* | -0.83 *0.76* | -2.04 *0.67* | -1.87 *0.96* | -1.25 *0.75* |
| | 25 µl | 90.79 *0.51* | -1.21 *0.74* | -2.13 *0.79* | -1.29 *0.89* | -0.92 *0.73* |
| | 50 µl | 90.87 *0.62* | -0.63 *0.70* | -1.38 *0.55* | -2.04 *0.90* | -2.29 *1.26* |
| Speed of Attention (summed msecs) | placebo | 1054.8 *24.13* | 15.55 *12.96* | 31.08 *14.55* | 57.44 *21.01* | 33.66 *14.34* |
| | 25 µl | 1059.7 *26.54* | -0.16 *12.96* | 29.47 *22.97* | 21.24 *16.01* | 17.10 *14.43* |
| | 50 µl | 1088.3 *48.14* | -3.40 *13.68* | 3.17 *42.70* | 31.48 *27.53* | -3.77 *40.67* |

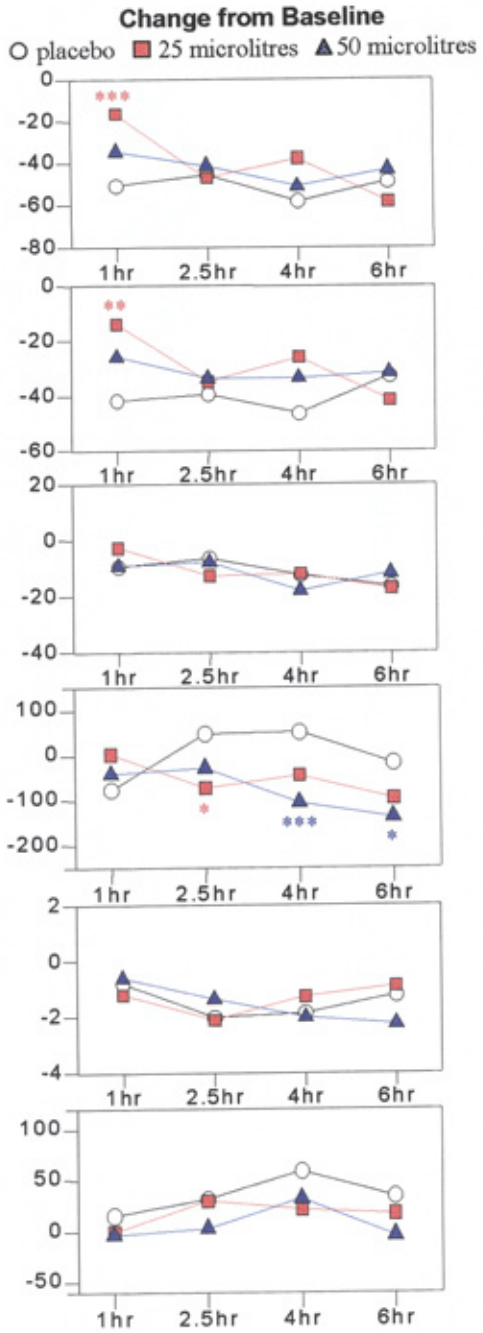


*Figure 9.1. Effects off Salvia lavandufoliae essential oil (25 µl and 50 µl) and placebo on cognitive measures, 'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Accuracy of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005 compared to the corresponding placebo score).Units are as per the table*

respectively), and 6 hours post-dose ([$t$(138) = 2.05, $p$ = 0.042].and [$t$(138) = 1.99, $p$ = 0.048] respectively). Similarly, while the 50 µl dose was associated with increased speed on the spatial memory task at 2.5 hours [$t$(138) = 3.5, $p$ = 0.0006] and 4 hours [$t$(138) = 2.03, $p$ = 0.044], both doses evinced faster performance at 6 hours post-dose ([$t$(138) = 2.17, $p$ = 0.03] and [$t$(138) = 3.7; $p$ = 0.0003] respectively). There was also a single increase in speed associated with the 25 µl dose on the numeric working memory task at 2.5 hours post-dose [$t$(138) = 2.09, $p$ = 0.039].

***Quality of* Attention factor**

There were no significant differences on the 'Quality of Attention' factor, or the measures that load onto it.

**Speed of Attention factor**

There were no significant differences on this factor. There was one single significant improvement in speed on the choice reaction time task for the 25 µl dose of sage at 4 hours post-dose [$t$(138) = 3.29, $p$ = 0.001]. It seems likely that this represents a chance fluctuation in performance.

**Serial Subtractions**

***Serial Threes***

There were significant differences on both the number of responses and errors on the Serial threes task. Following 50 µl of sage participants speed of performance improved significantly at the 6 hour testing session [t(138) = 3.44, p = 0.0008], in comparison to a decline for placebo. There was also a strong trend in the same direction for the 25 µl condition [t(138) = 1.93, p = 0.055]. In contrast to this the 50 µl condition evinced more errors at 2.5 hours post-dose [t(138) = 2.68, p = 0.008], and both doses were associated with more errors at 4 hours

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Serial 3s Responses | *placebo* | 59.46 *3.95* | 4.33 *1.76* | 5.54 *2.56* | 5.38 *2.06* | 1.71 *2.64* |
| | *25 µl* | 60.92 *2.98* | 1.83 *1.68* | 6.00 *2.33* | 4.54 *2.93* | 5.63 *2.89* |
| | *50 µl* | 57.58 *4.38* | 7.04 *1.66* | 6.33 *1.94* | 5.92 *1.63* | 8.67 *1.96* |
| Serial 3s Errors | *placebo* | 4.83 *1.05* | -0.04 *0.90* | -0.83 *0.87* | -2.33 *2.07* | -0.79 *0.83* |
| | *25 µl* | 4.42 *0.88* | 0.88 *0.90* | 0.38 *0.56* | 1.00 *1.20* | 0.50 *0.59* |
| | *50 µl* | 2.58 *0.86* | 0.96 *0.90* | 2.08 *1.08* | 2.58 *0.86* | 1.17 *0.91* |
| Serial 7s Responses | *placebo* | 35.79 *2.31* | 1.50 *1.04* | 1.63 *1.23* | 0.21 *1.18* | -2.75 *1.31* |
| | *25 µl* | 33.33 *2.09* | 1.67 *1.05* | 2.38 *1.43* | 3.08 *1.29* | 2.38 *1.34* |
| | *50 µl* | 34.71 *2.61* | 3.33 *0.94* | 1.42 *1.53* | 2.63 *1.04* | 3.00 *1.51* |
| Serial 7s Errors | *placebo* | 3.63 *0.74* | 0.38 *0.78* | 0.75 *0.70* | -0.38 *0.96* | 0.08 *0.90* |
| | *25 µl* | 3.92 *0.49* | -0.17 *0.78* | -0.63 *0.53* | -0.13 *0.49* | 0.04 *0.61* |
| | *50 µl* | 3.54 *0.41* | 0.42 *0.78* | 0.58 *0.52* | 0.33 *0.66* | 1.08 *1.22* |

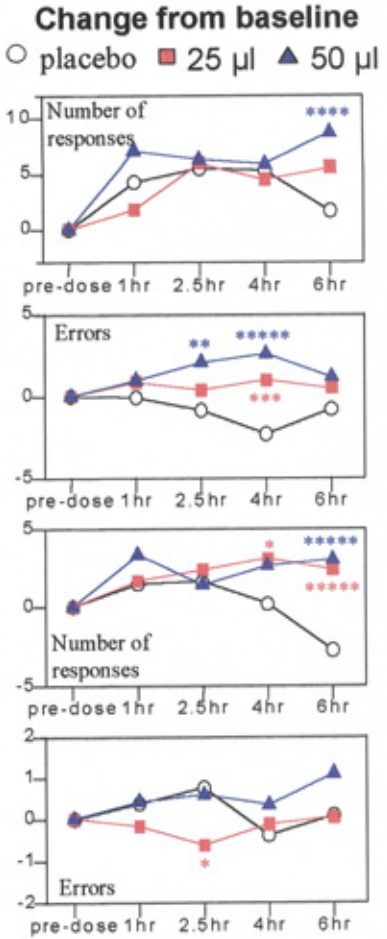


*Figure 9.2. Effects of Salvia lavandulaefolia essential oil (25 µl and 50 µl) and placebo on serial subtraction performance. The table presents means (with standard errors in italics) of baseline and change from baseline scores for each treatment in terms of total reponses and errors on both the Serial Threes and Serial Sevens tasks. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01 compared to the corresponding placebo score).*

post-dose ( 25 µl [t(138) = 3.07, p = 0.003], and 50 µl [t(138) = 4.52, p = 0.00001]). While the increase in errors following the 50 µl dose has to be viewed with caution in light of the significant baseline reduction in errors for this condition, and the possibility that the increase in errors therefore represents a regression to the mean, it is interesting to note that pre-dose scores for 25 µl were largely indistinguishable from placebo.

***Serial Sevens***

Performance was improved following both doses of sage on the Serial Sevens task. In comparison to placebo participants generated more subtractions above baseline performance at 4 hours post-dose following 25 µl [t(138) = 2.02, p = 0.045], and at 6 hours post-dose following both doses of sage (25 µl [t(138) = 3.6, p = 0.0004], and 50 µl [t(138) = 4.05, p = 0.00009]). There was also a single significant reduction in errors following 25 µl at 2.5 hours post-dose [t(138) = 2.21, p = 0.029].

Mean raw and change from baseline serial subtraction scores for each condition across each session are displayed in Figure 9.2.

**Bond-Lader Mood Scales**

*'Alert'* Participants subjective ratings showed that, in comparison to baseline ratings, they rated themselves as more alert than placebo following ingestion of 50 µl of sage at all testing sessions, with this reaching significance at the 2.5 hour [t(138) = 2.34, p = 0.02], and 4 hour [t(138) = 2.58, p = 0.01] testing sessions.

*'Calm'* Participants also scored higher on the 'calm' factor than at the baseline testing session, in comparison to placebo, following 50 µl of sage at all testing sessions ( 1 hour [t(138) = 2.22,

p = 0.028], 2.5 hours [t(138) = 3.46, p = 0.0007], 4 hours [t(138) = 2.6, p = 0.01] and 6 hours post-dose [t(138) = 3.52, p = 0.0006]). Similarly, having ingested 25 µl of sage participants rated themselves as having become more 'calm' than after taking the placebo at all time points, with this reaching significance at 2.5 hours [t(138) = 3.01, p = 0.003] and 6 hours post-dose [t(138) = 2.74, p = 0.007].

*'Content'* Participants also rated themselves as having become significantly more content, in comparison to placebo, in the 50 µl condition at the 4 hour [t(138) = 2.96, p = 0.0036], and 6 hour [t(138) = 2.3, p = 0.023] testing sessions.

Mean raw and change from baseline scores on the 'alert', 'content' and 'calm' factors derived from the Bond-Lader visual analogue scales for each condition across each session are displayed in Figure 9.3.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 2.5 hours | 4 hours | 6 hours |
|---|---|---|---|---|---|---|
| 'Alert' | placebo | 58.18 3.76 | 4.38 2.64 | 2.91 2.39 | 1.05 3.33 | 5.02 3.01 |
| | 25 µl | 56.44 4.46 | 6.11 2.85 | 5.27 3.50 | -0.26 3.74 | 1.89 4.98 |
| | 50 µl | 58.23 3.89 | 8.38 2.98 | 9.44 2.67 | 8.25 3.02 | 8.79 4.48 |
| 'Calm' | placebo | 68.77 2.64 | -6.00 3.12 | -15.44 4.06 | -9.88 3.80 | -13.69 4.04 |
| | 25 µl | 63.33 3.60 | -4.33 3.26 | -2.19 4.08 | -4.44 5.17 | -1.65 4.49 |
| | 50 µl | 61.92 4.38 | 3.77 3.25 | -0.23 3.71 | 1.60 3.91 | 1.77 4.47 |
| 'Content' | placebo | 70.51 3.18 | -0.61 2.18 | 0.58 1.45 | -2.98 2.15 | -2.36 2.60 |
| | 25 µl | 73.66 2.73 | 1.50 2.39 | -2.17 3.19 | -6.31 3.08 | -1.59 1.87 |
| | 50 µl | 69.46 3.37 | 1.75 2.17 | 4.32 3.36 | 5.40 3.25 | 4.12 4.77 |

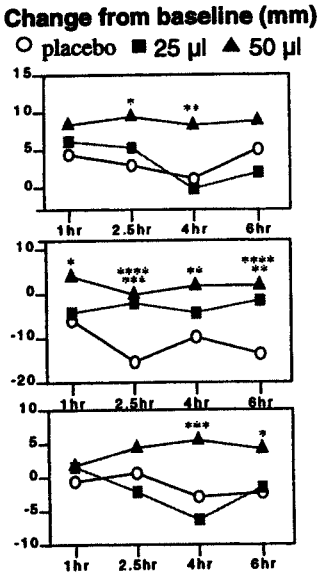


*Figure 9.3. Effects of Salvia lavandulaefolia essential oil (25 µl and 50 µl) and placebo on Bond-Lader mood scale factor scores; 'Alert', 'Content', and 'Calm'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each treatment. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001 compared to the corresponding placebo).*

## 9.4. Discussion

Both doses of *S. lavandulaefolia* resulted in improved performance, in comparison to placebo, on all three elements of the testing battery employed in the current study.

Improved performance on the primary cognitive measures derived from the CDR battery was restricted to memory performance, with specific improvements for the lowest dose (25 µl) on the 'Secondary Memory' factor at the 1 hour post-dose testing session, and on the 'Speed of Memory' factor at the 2.5 hour testing session. The 50 µl dose was associated with improved 'Speed of Memory' at both the 4 and 6 hour testing sessions. Reference to the single task outcomes showed that the sage oil conditions evinced improved performance on a number of tasks, but that again these were almost exclusively restricted to enhancement for both doses on the component tasks making up the 'Secondary Memory' and 'Speed of Memory' factors. It is notable that all of the significant changes on all of the tasks from the CDR battery reflected an improvement in performance.

On the serial subtraction tasks both doses of sage resulted in sustained improvement in the speed of performance, with the 50 µl dose resulting in more subtractions above baseline performance than placebo at the last (6 hour) testing session on both Serial 3s and Serial 7s. Similarly, the 25 µl dose resulted in more subtractions above baseline on the Serial 7s task at both the 4 hour and 6 hour testing sessions. Whilst accuracy of performance gradually improved for the placebo condition over the course of the first four testing sessions on the Serial 7s, the opposite was true for both sage conditions, with significantly more errors made, in comparison to placebo, at the 2.5 hour time point for 50 µl, and at the 4 hour time point for both doses. This last finding has to be viewed in the context of significantly reduced errors for the higher dose at the pre-dose baseline testing session, and improved accuracy of performance for the lower dose at 2.5 hours on the Serial 3s task. It also remains an intriguing possibility that both increased speed of performance and the reduction in accuracy on this task represents a

consequence of a more relaxed mood, in particular in terms of increased 'calmness' for both doses and increased 'contentedness' for the higher dose.

The improvements in mood seen for both doses of sage are possibly the most striking findings of the current study. This effect was most pronounced for the higher dose, which evinced greater enhancement than both other conditions on each factor of the Bond-Lader scales at each time point. These improvements were significantly above those for placebo at the 2.5 and 4 hour sessions on the 'alert' factor, at all time points on the 'calm' factor, and during the latter two testing sessions for the 'content' factor. Whilst ratings for the 25 µl dose were largely indistinguishable from placebo on the 'alert' and 'content' factors, this dose too was associated with sustained improvements in ratings on the 'calm' factor, in comparison to placebo, which reached significance at both 2.5 hours and 6 hours post-dose.

This overall pattern of results accords well with the properties traditionally attributed to *Salvia* species. Examples of references to the effects of the herb from 16$^{th}$ and 17$^{th}$ century pharmacopoeias include those by Gerard, in 1597, who suggests that *'It is singularly good for the head and brain and quickeneth the nerves and memory'*. Similarly, Culpepper , writing in 1652 in his authoritative 'Complete Herbal', notes that *'It also heals the memory, warming and quickening the senses'* (Perry *et al,* 1999).

To a great extent the pattern of cognitive modulation also agrees with recent identification of the *in vitro* cholinergic properties of sage, and the analysis of the *S. lavandulaefolia* undertaken for the current study. The demonstration of AChE inhibitory properties for the essential oil can certainly accommodate the improvements seen for both doses of sage on the 'Secondary Memory' and 'Speed of Memory' factors, and their component single task outcomes. Similarly, increased speed on the serial subtraction tasks can be viewed within the context of cholinergic modulation of central executive resources (Rusted and Warburton, 1991). Alternatively, the improvements evinced here could be seen in the light of recent demonstrations of oestrogenic properties for *S. lavandulaefolia* essential oil (Perry *et al,* 2001) and the attendant possibility of wide ranging CNS effects (Shepherd, 2001). In light of the complex composition of the

essential oil (in our own GCMS analysis there were a huge number of unidentified constituents), it is unlikely that any effects are attributable to one mechanism in isolation, and are more likely to reflect the additive or synergistic effects of a number of different components and/or mechanisms. Particularly pertinent to this point is the striking pattern of mood elevation, with the higher dose of sage resulting in increased ratings of alertness, calmness and contentedness. When both the cognitive and mood effects of the herb are considered it becomes increasingly unlikely that they are as a result of one simple mechanism.

The initial driving force behind the investigation of the cognitive effects of *Salvia* species was the possibility that they may constitute a natural, effective and safe treatment for the 'cholinergic' cognitive decrements associated with Alzheimer's disease (Perry *et al,* 1996; Perry *et al,* 1998). Specifically, treatments that inhibit AChE, retarding the catabolism of acetylcholine, and therefore resulting in increased synaptic availability of the neurotransmitter, have been shown to improve memory function in young and aged healthy human cohorts, and are currently the only widely used treatment for Alzheimer's disease (Amenta *et al,* 2001). Interestingly, Galantamine, a plant (daffodil and snow drop) derived AChE inhibitor has recently joined the small rank (Tacrine, Donepezil, Rivastigimine) of approved treatments for Alzheimer's disease (Parys, 1998). The essential oil utilised in the current study was shown to inhibit acetylcholinesterase, a property that has been demonstrated to occur *in vivo* in selected brain areas of rodents (Perry *et al,* 2002). Furthermore, there are no reports of negative side effects associated with *Salvia lavandulaefolia* (or *S. officinalis*) despite usage spanning hundreds, if not thousands, of years. Certainly the results evinced here provide a first demonstration in humans that this plant may well have cognition enhancing properties, and it is interesting to note that this beneficial modulation of mood and cognitive performance followed single doses of *S. lavandulaefolia,* and was evinced in a cohort of healthy young participants, who presumably have no cholinergic deficits.

Whilst amelioration of cognitive decline would undoubtedly be of benefit to sufferers from dementia, the antioxidant and anti-inflammatory properties of the plant extract (Perry *et al,*

2001) may well also confer long-term advantages in the pathogenesis of the disease. Similarly, the mood enhancing properties of the herb may well, in themselves, have applications in the treatment of advanced dementia, in which disturbed mood and agitation feature as a major problem.

The possibility that the positive behavioural effects demonstrated in the current study could also only be augmented by chronic regimens, and in cohorts suffering from age or disease related declines in functioning, deserves serious investigation.

# CHAPTER 10. MODULATION OF MOOD AND COGNITIVE PERFORMANCE FOLLOWING ACUTE ADMINISTRATION OF *MELISSA OFFICINALIS* (LEMON BALM)

## 10.1. Introduction

*Melissa Officinalis* has a recorded medicinal history spanning more than 2000 years (Koch-Heitzmann and. Schultze, 1988). Early European usage includes medieval recommendations by Paracelsus (1493-1541) that balm would completely revivify a man, and indication for *"all complaints supposed to proceed from a disordered state of the nervous system"* (Grieve, 1931). Several herbal Apothecaries of the time also attributed balm tea not only with general beneficial effects upon the brain, but also with specific mnemonic improvements (Coghan, 1584; Evelyn, 1699).

Contemporary reports stress the sedative, spasmolytic, and antibacterial effects of ingestion of *Melissa officinalis*, with indications encompassing nervous disorders including the reduction of excitability, anxiety, and stress, gastro-intestinal disorders and sleep disturbance (Bisset and Wichtl, 1994; Kommission E Monograph, 1984). In keeping with its long history of safe usage no side effects have so far been reported (Wong *et al,* 1998).

*Melissa officinalis* is predominantly sold in combination with other herbs, with, as an illustration, 49 products containing Lemon-balm in the German pharmaceutical industry's current 'Rote Liste' (2001) drug catalogue.

A number of studies involving rodents suggest specific 'calming' or sedative effects. Examples include a reduction in spontaneous movement demonstrated in mice as a consequence of both the whole volatile oil of melissa and the individual isolated terpenes (Wagner and Sprinkmeyer, 1973). Similarly, reductions in behavioural parameters in mice on both familiar and non-familiar environment tests were elicited by an hydroalchoholic extract of melissa. An inverted U shaped dose response was evident with the greatest effect following 25mg/kg (dose range 6-

100mg/kg). The plant extract also increased pentobarbital induced sleep parameters (Soulimani *et al,* 1991).

Whilst no studies have looked at the effects on humans of the ingestion of melissa by itself, several have investigated the effects of a valerian/melissa combination on sleep quality, with, for example, similar improvements demonstrated as those associated with 0.125mg of Triazolam in poor sleepers (Dressing *et al,* 1992), and significant improvements in quality of sleep, in comparison to placebo, during 30 days treatment with 600 mg/day of a combination including the *M. officinalis* extract utilized in the current study (Cerny and Schmid, 1999).

A single, recent, double-blind, placebo-controlled study (Ballard *et al,* 2002) also examined the effect of *M. officinalis* essential oil aromatherapy on 3rd party ratings of agitation and 'quality of life' of 71 patients suffering from severe dementia. Following 4 weeks treatment patients in the active treatment group were rated, in comparison to the placebo group, as less agitated, less socially withdrawn, and as engaged in more time spent in constructive activities.

Behavioural consequences such as these could be attributable to a number of possible active components of the dried leaf and essential oil of the herb. Constituents that have been identified include a number of monoterpenoid aldehydes (including Citronellal, Neral and Geranial), (Carnat *et al,* 1998), flavonoids and polyphenolic compounds (most notably rosmarinic acid) (Carnat *et al,* 1998; Hohmann *et al,* 1999) and monoterpene glycosides (Mulkens *et al,* 1985).

It has been suggested, on the basis of a retrospective review of the historical role of a number of European plant species in the enhancement of memory, that *M. officinalis*, and another plant in the Labiatae family, *Salvia officinalis (*Sage), might potentially provide novel natural treatments for Alzheimer's disease (Perry *et al,* 1999). This approach has generated research showing that *Melissa officinalis* exhibits central nervous system acetylcholine receptor activity, with demonstrations of both nicotinic (Perry *et al,* 1996; Wake *et al,* 2000) and muscarinic (Wake *et al,* 2000) binding properties. In the case of the latter study, six separate accessions of Melissa leaf elicited markedly different proportions of binding to the two acetylcholine receptor subtypes in human occipital cortex tissue, with $IC_{50}$ concentrations ranging from 0.08 mg to 3.8

mg/ml for the displacement of [$^3$H]-(N)-nicotine from nicotinic receptors, and from 0.5 to >5 mg/ml for the displacement of [$^3$H]-(N)-scopolamine from muscarinic receptors. These properties might provide a potential treatment for the cholinergic disturbances in Alzheimer's disease. Additionally, demonstrations of antioxidant activity (Hohmann *et al,* 1999; Mantle *et al,* 2000) suggest that melissa may also provide some protection against the putative aetiological free radical damage in dementia.

Given its long history as a memory enhancer, contemporary usage as a mild sedative, sparse but suggestive animal studies, and the recent delineation of possible specific CNS neurotransmitter effects, it is possible that *Melissa Officinalis* may exert beneficial effects on the cognitive performance of humans.

The present study investigated the dose response relationship, and time course, of possible changes in mood and cognitive performance in healthy young volunteers following single doses of *Melissa officinalis*. The mood and cognitive outcome measures were as described in Chapter 2 (CDR battery and Bond-Lader mood scale) and Chapter 5 (Serial subtractions). Nicotinic and muscarinic binding properties for the specific *Melissa officinalis* extract were investigated using the *in vitro* methods utilised by Wake *et al,* (2000).

## 10.2. Materials and Methods

### The *Melissa officinalis* preparation

A standardised, commercial extract of *Melissa officinalis* prepared by Pharmaton SA (Lugano, Switzerland) was utilised in the current study. Standardisation and conformity of the extract is assured by strict in-process controls during manufacture and complete analytical control of the resulting dry extract. The production method involves dried leaves of *Melissa officinalis* being reduced to fragments and extracted up to exhaustion in a 30:70 methanol-water mixture. The resultant liquid extract is evaporated and homogenised to yield a soft extract, to which inert processing agents (dried glucose syrup and colloidal anhydrous silicon dioxide to 7% and 3% of the final dried weight respectively) are added. This mixture is homogenised and taken to dryness, ground, mixed and sieved.

### Cholinergic receptor binding and chemical analysis

In order to provide a valid comparison with previous studies assessing the cholinergic receptor binding properties of *Melissa officinalis* leaf (Wake *et al,* 2000), the $IC_{50}$ concentrations for the displacement of [$^3$H]-(N)-nicotine from nicotinic receptor, and [$^3$H]-(N)-scopolamine from muscarinic receptors were established in human occipital cortex tissue, using an identical extraction and receptor methodology to that previously used (for details see Wake *et al,* 2000). The melissa extract was also analysed using Gas Chromatograph Mass Spectroscopy (GCMS) for terpene constituents. This analysis was undertaken at the MRC Centre for Development in Clinical Brain Ageing, Newcastle General Hospital.

### Participants

15 female and 5 male undergraduate volunteers (mean age 19.2 years, range 18-22 years) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North

Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Habitual smokers were excluded from the study. Of the 20 participants 2 were occasional, light, social smokers (average consumption < 2 cigarettes a day in both cases) and they agreed to abstain from smoking from rising on the day of the study until after completion of testing. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

**Cognitive Measures**

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive factors were as described in detail previously in Chapter 2 (section 2.2. pages 87-93). Table 10.1 presents the baseline and change from baseline mean scores from the individual tasks within the battery in order of completion.

**Subjective mood measure**

The 16 Bond-Lader Visual Analogue Scales (Bond and Lader 1974). were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

**Serial subtraction tasks**

Modified computerised versions of the Serial Threes and Serial Sevens tasks, as described in detail in Chapter 5 (pages 141-142) were employed.

**Treatments**

On each study day participants received six capsules of identical appearance, each containing either an inert placebo or 150 mg of *Melissa officinalis* extract. Depending on the condition to which they were allocated on that particular day the combination corresponded to a dose of either 0 (placebo), 300 mg, 600 mg, or 900 mg of *Melissa officinalis* extract.

**Procedure**

The procedure was identical to that described in Chapter 2 (section 2.2. page 94). Each participant was required to attend a total of five study days that were conducted seven days apart, to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment on visits 2 to 5. Further testing sessions began at 1 hour, 2.5 hours, 4 hours and 6 hours following consumption of the day's treatment.

Each testing session comprised completion of the Bond-Lader Visual Analogue Scales, the CDR test battery, and finally the Serial 3s and Serial 7s computerised subtraction tasks.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, and the individual task outcome measures making up the factors, were analysed as 'change from baseline' using the SAS statistical package. The initial analysis was made using the general linear models procedure (PROC GLM). Following the recommendations of Keppel (1991) the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three *M. officinalis* conditions (300 mg, 600 mg and 900 mg) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

The three mood outcomes derived from the Bond-Lader scales were analysed using within subjects Analyses of Variance (Minitab) with planned comparisons as per the above.

## 10.3. Results

### Cholinergic receptor binding analysis

The $IC_{50}$ concentrations for nicotinic and muscarinic receptor binding to human occipital cortex tissue of extracts of the encapsulated material were 11 mg/ml and 4 mg/ml respectively.

It was not possible to extract sufficient material from the *M. officinalis* preparation for GCMS analysis of terpene content.

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 300 mg, 600 mg, and 900 mg *M. officinalis*) for each primary outcome (cognitive factor scores, serial subtraction scores, and mood scale scores) were subjected to a one-way, repeated measures, ANOVA. There were no significant differences on any measure.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are presented in Table 10.1. Results on individual task outcomes are described in relationship to the overall factor to which they contribute below (memory task results are presented with either the 'Secondary Memory' or 'Working Memory' factors to which they contribute).

### Cognitive factor outcome measures

Mean raw baseline scores and change from baseline factor scores for each condition across each session are represented in the tables and graphs of Figure 10.1.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Immediate Word Recall (% accuracy) | placebo | 49.00 4.16 | -2.33 3.32 | -3.17 4.25 | -0.50 4.61 | -2.67 3.93 |
| | 300mg | 47.00 3.32 | -0.83 3.14 | 3.50 3.49 | -1.33 4.18 | -2.67 3.72 |
| | 600mg | 50.17 3.79 | -1.83 2.92 | -2.67 2.58 | -4.00 3.89 | -3.33 3.48 |
| | 900mg | 47.83 3.54 | -3.50 5.11 | -8.50 3.46 | -5.33 5.03 | -4.67 3.54 |
| Simple Reaction time (msecs) | placebo | 267.57 7.24 | 4.99 8.56 | 8.87 6.91 | 5.15 10.30 | 10.58 9.13 |
| | 300mg | 266.19 9.56 | 4.55 4.86 | 2.75 5.65 | 18.93 10.10 | 31.92 17.79* |
| | 600mg | 263.17 6.98 | 11.95 6.70 | 6.51 7.87 | 15.76 9.41 | 19.65 5.68 |
| | 900mg | 262.86 4.68 | 17.33 9.16 | 14.96 8.84 | 21.23 11.05 | 29.07 13.34* |
| Digit Vigilance Accuracy (%) | placebo | 96.67 1.03 | -1.33 1.72 | 0.67 1.27 | -2.00 1.88 | 1.67 1.27 |
| | 300mg | 97.67 0.88 | -1.33 1.33 | -2.00 1.46 | -1.33 1.42 | -0.33 1.13 |
| | 600mg | 94.67 1.42 | 3.33 1.23**** | 2.00 1.46 | 3.00 1.23***** | 3.00 1.41 |
| | 900mg | 97.00 1.13 | -0.67 1.07 | 0.00 1.45 | -0.33 1.23 | -0.67 1.60 |
| Digit Vigilance False alarms (number) | placebo | 0.45 0.15 | 0.40 0.24 | 0.15 0.28 | 0.00 0.19 | 0.10 0.14 |
| | 300mg | 0.60 0.20 | -0.05 0.21 | -0.05 0.21 | 0.00 0.27 | 0.00 0.30 |
| | 600mg | 0.60 0.15 | -0.20 0.20* | 0.15 0.27 | -0.20 0.21 | -0.30 0.22 |
| | 900mg | 0.65 0.13 | -0.30 0.16** | 0.20 0.25 | -0.15 0.20 | -0.10 0.20 |
| Digit Vigilance Reaction time (msecs) | placebo | 396.68 7.91 | -1.87 7.48 | 1.23 6.89 | 14.36 9.83 | 12.02 8.16 |
| | 300mg | 397.20 6.72 | 1.46 8.21 | -0.32 7.47 | 3.91 7.27 | 12.20 7.49 |
| | 600mg | 396.29 6.99 | 0.78 9.35 | 1.86 8.16 | 7.47 9.66 | 14.26 10.42 |
| | 900mg | 398.63 6.63 | 3.93 7.63 | 5.56 8.75 | 2.52 7.32 | 20.40 8.83 |
| Choice reaction time accuracy (%) | placebo | 95.00 1.00 | -1.60 0.75 | -2.80 0.96 | -2.20 1.04 | -2.60 1.13 |
| | 300mg | 94.70 0.91 | 0.50 0.82* | -1.70 1.08 | 0.30 1.03** | -2.90 1.01 |
| | 600mg | 94.10 1.24 | 0.20 1.12 | -0.70 1.34* | -1.00 0.98 | -1.00 1.14 |
| | 900mg | 94.10 0.90 | -0.80 1.22 | -0.60 0.88* | -1.50 1.17 | 0.40 1.00*** |
| Choice reactionTime (msecs) | placebo | 425.04 12.62 | 4.56 13.15 | -5.81 7.69 | -3.21 7.50 | -2.59 9.80 |
| | 300mg | 437.94 19.91 | -9.39 8.37 | -17.87 8.38 | -13.22 10.15 | -10.27 17.96 |
| | 600mg | 418.45 8.82 | 3.23 5.78 | -2.61 8.65 | 8.27 9.40 | 9.74 7.37 |
| | 900mg | 431.63 12.12 | 1.57 9.09 | 2.58 11.03 | -4.00 11.10 | -4.93 9.20 |
| Spatial Memory (%>chance) | placebo | 85.31 5.05 | 6.50 5.20 | 2.31 5.97 | 1.75 5.50 | 3.56 6.50 |
| | 300mg | 91.56 2.74 | -1.06 2.54 | -10.31 6.38*** | 0.25 2.47 | -5.56 5.48* |
| | 600mg | 93.94 1.21 | -1.25 1.74 | -10.75 3.61*** | -0.44 1.85 | -4.19 1.65 |
| | 900mg | 92.25 1.60 | -4.25 3.91* | -6.69 4.59* | -2.50 2.17 | -6.38 3.69* |
| Spatial memory Reaction time (msecs) | placebo | 603.16 28.60 | -17.33 27.98 | -52.78 23.46 | -48.77 21.52 | -61.10 24.94 |
| | 300mg | 595.81 30.12 | -16.51 23.23 | -39.30 23.39 | -44.72 28.12 | -60.22 20.27 |
| | 600mg | 592.01 28.91 | -16.71 15.85 | -28.33 19.69 | -36.91 21.97 | -7.96 25.58 |
| | 900mg | 599.03 29.68 | -35.61 22.24 | -27.07 20.13 | -20.11 20.46 | -45.01 20.82 |
| NumericWork'g Memory (%>chance) | placebo | 84.33 2.66 | -2.11 2.75 | -1.00 2.81 | -4.11 2.51 | -6.55 2.81 |
| | 300mg | 87.00 2.58 | -6.89 2.23 | -1.22 1.70 | -3.56 1.75 | -4.22 1.64 |
| | 600mg | 86.00 2.38 | -1.44 3.14 | -3.00 2.59 | -3.44 2.36 | -2.89 1.66 |
| | 900mg | 86.00 2.64 | -5.67 1.83 | -4.89 1.85 | -5.89 2.79 | -7.11 2.50 |
| Numeric Working Memory Reaction Time (msecs) | placebo | 515.88 20.52 | 5.17 9.23 | -14.86 8.48 | -8.85 6.22 | -23.80 12.41 |
| | 300mg | 523.64 17.51 | -6.09 11.95 | -0.01 10.82 | -9.01 8.79 | -22.12 13.05 |
| | 600mg | 548.97 22.47 | -11.09 10.32 | -11.98 11.70 | -37.07 9.00 | -20.15 9.70 |
| | 900mg | 522.74 18.28 | 6.56 9.17 | 4.28 12.31 | -3.99 14.21 | -31.76 14.61 |
| Delayed Word Recall (% accuracy) | placebo | 36.67 3.03 | -15.33 2.95 | -13.50 3.28 | -13.67 2.56 | -17.67 3.33 |
| | 300mg | 37.50 3.00 | -9.50 2.42 | -12.33 4.24 | -14.50 4.27 | -15.83 3.26 |
| | 600mg | 36.17 3.24 | -10.33 3.44 | -8.33 2.30 | -11.00 3.14 | -17.00 2.81 |
| | 900mg | 36.67 3.57 | -11.00 3.60 | -17.83 3.02 | -23.00 4.72* | -16.83 3.80 |
| Word Recognition (%>chance) | placebo | 50.33 5.81 | 5.00 3.90 | 6.33 4.57 | 5.67 6.95 | 2.33 6.16 |
| | 300mg | 59.33 4.61 | -2.67 3.76 | -4.33 5.24 | -10.67 4.85*** | -8.33 5.34 |
| | 600mg | 65.71 5.18 | -9.37 4.66** | -14.04 4.74***** | -16.71 6.00***** | -20.04 5.42***** |
| | 900mg | 53.67 5.87 | 4.33 5.67 | -9.58 6.39*** | -2.67 4.06 | -6.27 4.60 |
| Word Recognition Reaction time (msecs) | placebo | 680.21 37.77 | -18.69 35.35 | -15.97 38.67 | -3.79 41.75 | -37.96 37.87 |
| | 300mg | 667.84 21.92 | 23.70 22.81 | 12.58 22.52 | -2.88 19.46 | -0.80 17.08 |
| | 600mg | 663.26 22.29 | 10.25 17.85 | -0.49 21.12 | -4.05 19.63 | 9.70 23.88* |
| | 900mg | 664.99 22.67 | 1.52 20.25 | 24.73 18.00 | 6.20 21.79 | -6.16 22.28 |
| Picture Recognition (%>chance) | placebo | 66.50 5.67 | -0.75 6.01 | -1.50 7.45 | -0.50 6.58 | -12.50 8.19 |
| | 300mg | 68.50 5.59 | -6.50 4.91 | -10.25 4.35* | -5.25 3.49 | -13.00 3.67 |
| | 600mg | 69.00 4.27 | -4.25 4.39 | -5.25 4.52 | -14.50 4.76*** | -0.25 3.65** |
| | 900mg | 67.75 4.36 | -8.00 3.25 | -8.00 3.02 | -11.50 3.48* | -13.75 4.17 |
| Picture recognit'n Reaction time (msecs) | placebo | 741.88 25.36 | -7.84 14.85 | -10.32 18.62 | 2.92 17.00 | -14.37 19.19 |
| | 300mg | 738.49 25.81 | -0.45 21.00 | 11.19 16.95 | -7.11 15.22 | -20.44 25.27 |
| | 600mg | 748.99 23.03 | 12.56 17.00 | 0.35 20.83 | 2.33 20.42 | 9.53 16.11 |
| | 900mg | 741.83 26.46 | 13.77 14.37 | 9.34 21.55 | 17.31 23.83 | 0.40 17.37 |

*Table 10.1 Effects of M. officinalis on individual task outcome measures from the CDR battery,. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, ****,= 0.001,*****, = 0.0005 compared to placebo)*

***Quality of Memory measure***

Planned comparisons of change from baseline data revealed significant decrements in the accuracy of memory task performance, in comparison to placebo, for both 600mg and 900 mg of *M. officinalis* at 2.5 hours ([$t$(171) = 2.63, p = 0.009] and [t(171)=3.53, p = 0.0005] respectively), and at 4 hours post-dose [$t$(171) = 3.03; p = 0.0028] and [t(171)=3.01; p=0.0023] respectively).

***Secondary Memory Factor***

Whilst the highest dose alone evinced a decrement on the 'Secondary Memory' factor at the 2.5 hour testing session [t(171)=2.83; p=0.005], all three doses of M. officinalis resulted in significant impairment at the 4 hour testing session (300 mg [t(171)=2.01, p= 0.046], 600 mg [t(171)=3.29; p=0.0012], and 900 mg [t(171)=2.96; p=0.0035].

Comparisons of the individual task outcome scores suggest that the decrements were particularly apparent on performance of the word recognition task (% accuracy above chance). Performance was significantly disturbed at all time points for the 600 mg dose (1 hour [t(171)=2.61, p=0.009], 2.5 hours [t(171)=3.7, p=0.0003], 4 hours [t(171)=4.08, p=0.00007]and 6 hours [t(171)=4.07, p= 0.00007]. The 300 mg dose evinced a similar pattern with decrements that reached significance at 4 hours [t(171)=2.96, p=0.0035] with strong trends towards significant decrements at 2.5 [t(171)=1.94, p=0.054] and 6 hours [t(171)=1.94, p=0.054]. There was a single decrement associated with the 900 mg dose at the 2.5 hour testing session [t(171)=2.89, p=0.004].

Performance on the delayed picture recognition task (% accuracy above chance) was also significantly disturbed at 4 hours post-dose for both the 600 mg [t(171)=3.06, p=0.003] and 900 mg doses [t(171)=2.41, p=0.017], with a trend towards a decrement at 2.5 hours for the 300 mg dose [t(171=1.91, p=0.058].

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | | |
|---|---|---|---|---|---|---|
| | | | 1 hour | 2.5 hours | 4 hours | 6 hours |
| Quality of Memory (%X 6) | placebo | 372.15 17.20 | -9.03 13.17 | -10.52 14.39 | -11.36 19.68 | -33.49 20.95 |
| | 300mg | 390.90 15.55 | -27.45 10.76 | -34.95 14.03 | -35.06 12.00 | -49.62 10.28 |
| | 600mg | 400.98 13.49 | -28.48 8.85 | -44.04 9.29 | -50.09 12.30 | -47.70 9.63 |
| | 900mg | 384.17 14.75 | -28.08 14.99 | -55.49 13.41 | -50.89 13.05 | -55.01 11.78 |
| Secondary Memory (%X 4) | placebo | 202.50 13.71 | -13.42 11.14 | -11.83 12.62 | -9.00 15.48 | -30.50 16.54 |
| | 300mg | 212.33 12.66 | -19.50 9.10 | -23.42 13.53 | -31.75 12.31 | -39.83 8.46 |
| | 600mg | 221.04 11.99 | -25.79 8.43 | -30.29 7.69 | -46.21 11.31 | -40.62 8.59 |
| | 900mg | 205.92 13.14 | -18.17 12.51 | -43.92 11.50 | -42.50 11.70 | -41.52 9.12 |
| Working Memory (%X 2) | placebo | 169.65 6.63 | 4.39 6.44 | 1.31 7.42 | -2.36 6.86 | -2.99 7.92 |
| | 300mg | 178.56 4.74 | -7.95 3.72 | -11.53 6.83 | -3.31 2.71 | -9.78 5.22 |
| | 600mg | 179.94 2.97 | -2.69 3.75 | -13.75 4.78 | -3.88 3.05 | -7.07 2.40 |
| | 900mg | 178.25 3.62 | -9.92 4.97 | -11.58 4.92 | -8.39 3.38 | -13.49 5.10 |
| Speed of Memory (summed msecs) | placebo | 2541.1 92.35 | -38.69 41.89 | -93.93 63.18 | -58.49 53.35 | -137.2 68.23 |
| | 300mg | 2525.8 81.32 | 0.65 60.50 | -15.55 52.35 | -63.71 46.12 | -103.6 64.18 |
| | 600mg | 2553.2 85.00 | -4.99 40.32 | -40.44 55.43 | -75.70 48.99 | -8.87 52.50** |
| | 900mg | 2528.6 82.94 | -13.77 34.20 | 11.28 40.45* | -0.59 50.29 | -82.53 44.78 |
| Quality of Attention (%) | placebo | 90.55 0.91 | -1.80 0.95 | -1.25 0.63 | -2.00 1.05 | -0.65 0.96 |
| | 300mg | 90.70 0.65 | -0.30 0.77 | -1.70 0.95 | -0.45 0.89 | -1.60 0.77 |
| | 600mg | 89.05 0.86 | 1.80 0.78 | 0.40 1.04* | 1.05 0.84 | 1.15 0.98* |
| | 900mg | 90.05 0.66 | -0.40 0.71 | -0.50 0.76 | -0.75 0.80 | 0.00 0.85 |
| Speed of Attention (summed msecs) | placebo | 1089.3 22.37 | 7.69 24.94 | 4.30 15.76 | 16.30 19.67 | 20.01 18.74 |
| | 300mg | 1101.3 30.88 | -3.38 15.43 | -15.43 15.08 | 9.62 21.46 | 33.84 34.55 |
| | 600mg | 1077.9 16.90 | 15.96 13.83 | 5.76 15.68 | 31.50 23.10 | 43.65 15.79 |
| | 900mg | 1079.1 18.17 | 36.80 26.12 | 36.14 26.81 | 33.76 31.18 | 58.55 35.74 |

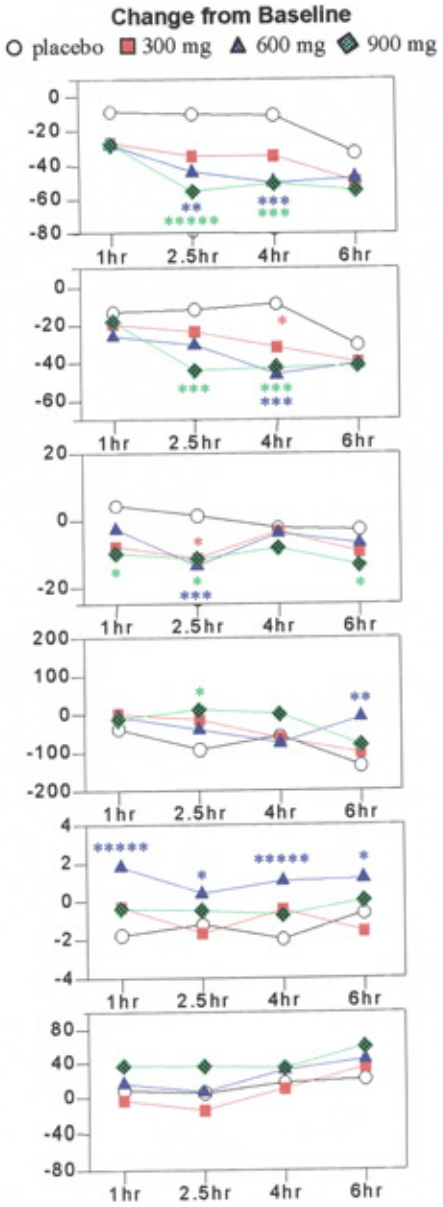


*Figure 10.1 .Effects of Melissa officinalis on the cognitive factors 'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Accuracy of Attention', and 'Speed of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of M. officinalis Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001; *****, p = 0.0005 compared to the corresponding placebo score). Graph units are as per the table.*

Both immediate and delayed word recall task performance were largely unaffected, with a single decrement in delayed word recall (900 mg at 4 hours [t(171)=2.55, p=0.012]). Given the nature of the statistical analysis it seems reasonable not to over-interpret isolated significant data points.

***Working Memory Factor***

All three doses of M. officinalis resulted in significant decrements on the 'Working Memory' factor. At the 1 hour post-dose testing session both 300 mg [t(171)=2.38, p=0.018] and 900 mg [t(171)=2.76, p=0.006] evinced significant reductions in change from baseline scores. This was also true for all three doses at 2.5 hours (300 mg [t(171=2.47, p=0.014], 600 mg [t(171)=2.9, p=0.0042], and 900 mg [t(171)=2.49, p=0.014], and for the 900 mg dose at 6 hours post-dose [t(171)=2.02, p=0.044].

Comparison of the individual task scores suggested that this effect was isolated to the spatial memory task (% accuracy above chance), on which measure performance was significantly impaired for 300 mg at 2.5 and 6 hours ([t(171)=2.89, p=0.004] and [t(171)=2.09, p=0.038] respectively), for 600 mg at 2.5 hours post-dose [t(171)=2.99, p=0.003], and for 900 mg at I hour [t(171)=2.47, p=0.014], 2.5 hours [t(171)=2.06, p=0.041] and 6 hours post-dose [t(171)=2.28,p=0.024].

***Speed of Memory factor***

Significant differences, in comparison to placebo, were restricted to a comparative slowing of performance for the 600 mg dose at 6 hour post-dose [t(171)=2.76, p=0.006] and the 900 mg dose at 2.5 hours post-dose [t(171)=2.26, p=0.025].

Single component task significant effects for the four tasks loading on this factor were restricted to a comparative slowing of performance on the delayed word recognition task for the 600 mg dose at 6 hours post-dose [t(171)=2.22, p=0.028].

***Speed of attention factor***

There were no significant differences for any dose on the 'Speed of Attention' factor. However, comparisons of the three component task measures revealed that there were decrements that were restricted to slowing of speed of performance on the simple reaction time task for 300 mg and 900 mg at 6 hours post-dose ([t(171)=2.36, p=0.02], and [t(171)=2.04, p=0.043] respectively).

***Quality of attention factor***

Performance was significantly improved for the 600 mg dose of *M. officinalis* at all time points (1 hour [t(171)=4.32, p=0.0001], 2.5 hours [t(171)=1.98, p=0.049], 4 hours [t(171)=3.66, p=0.0003], and 6 hours [t(171)=2.16, p=0.03]).

Inspection of the component measures revealed that whilst significant improvements on Digit vigilance accuracy were restricted to the 600 mg dose, with improvements at 1 hour [t(171)=3.35, p=0.001] and 4 hours post-dose [t(171)=3.59, p=0.0004], all doses evinced improvements on the choice reaction time task accuracy, with significant improvements for 300 mg at 1 hour [t(171)=2.2, p=0.029] and 4 hours post-dose [t(171)=2.62, p=0.01], for 600 mg at 2.5 hours post-dose [t(171)=2.2, p=0.029], and for 900 mg at 2.5 hours [t(171)=2.3, p=0.022], and 4 hours post-dose [t(171)=3.14, p=0.002]. Digit vigilance false alarms were also improved for both 600 mg and 900 mg at 1 hour post-dose ([t(171)=2.3, p=0.023] and [t(171)=2.68, p=0.008] respectively).

**Serial Subtractions**

Due to data capture errors the number of participants contributing to the serial subtraction tasks was reduced to 17 for Serial 3s, and 18 for Serial 7s.

***Serial Threes Task***

In comparison to placebo,there was a single reduction in responses for the 900 mg dose at the 4 hour testing session [t(144)=2.95, p=0.0037].

There were no significant differences in the number of errors on the Serial 3s task.

***Serial Sevens Task***

There were no significant differences on either number of responses or number of errors for any dose on the Serial 7s task.

**Bond-Lader visual analogue mood scales**

*'Alert'*: Planned comparisons revealed that the 900 mg dose of *M. officinalis* was associated with a significant reduction in scores at all testing sessions (1 hour [t(171) = 3.81, p = 0.0002], 2.5 hours [t(171) = 2.3, p = 0.023], 4 hours [t(171) = 2.53, p < 0.012], and 6 hours [t(171) = 3.73, p = 0.0003] respectively). The 300 mg dose resulted in a single significant reduction at the 6 hour testing session [t(171)=2.1, p=0.037].

*'Content'*: There was no modulation of the 'content' factor.

*'Calm':* In comparison to placebo, ratings on the 'calm' scale were significantly improved for both 300 mg and 900 mg at the first (1 hour) testing session ([t(171)=3.13, p=0.002] and [t(171)=2.36, p=0.019] respectively). The 300mg dose was also associated with an improvement on this scale at 2.5 hours post-dose [t(171)=2.21, p=0.028]

The effects of *M. Officinalis* on the mood measures are presented in the table and graphs of Figure 10.2

| Factor | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 2.5 hours | 4 hours | 6 hours |
|---|---|---|---|---|---|---|
| ALERT | *placebo* | 50.37 *3.85* | 9.01 *3.52* | 6.85 *3.98* | 10.58 *4.36* | 11.07 *4.30* |
| | *300mg* | 53.40 *4.26* | 6.63 *3.47* | 10.39 *4.60* | 7.06 *3.02* | 5.65 *3.59* |
| | *600mg* | 50.89 *4.70* | 5.14 *2.94* | 4.44 *3.48* | 6.15 *3.83* | 6.85 *3.46* |
| | *900mg* | 53.09 *3.84* | -0.83 *2.89* | 0.92 *3.97* | 4.07 *3.90* | 1.45 *4.34* |
| CONTENT | *placebo* | 63.87 *3.72* | -0.34 *3.27* | 2.28 *3.01* | 1.57 *3.92* | 2.88 *3.63* |
| | *300mg* | 68.29 *3.24* | 1.95 *1.93* | 1.18 *1.81* | -2.19 *2.15* | -1.24 *2.97* |
| | *600mg* | 62.87 *3.85* | 0.28 *3.03* | -0.93 *2.84* | 1.97 *2.81* | 3.10 *3.74* |
| | *900mg* | 61.22 *2.71* | 2.33 *3.60* | -1.13 *3.12* | 2.70 *2.72* | -0.72 *3.13* |
| CALM | *placebo* | 70.80 *2.91* | -12.53 *2.24* | -8.30 *3.07* | -10.98 *3.13* | -13.13 *3.64* |
| | *300mg* | 65.05 *3.80* | -2.25 *2.42* | -1.05 *2.30* | -5.93 *2.62* | -9.30 *2.94* |
| | *600mg* | 69.59 *3.42* | -7.04 *3.01* | -14.46 *3.85* | -11.29 *3.83* | -16.01 *4.57* |
| | *900mg* | 64.33 *2.96* | -4.80 *2.59* | -11.80 *3.95* | -8.73 *2.99* | -10.40 *2.52* |

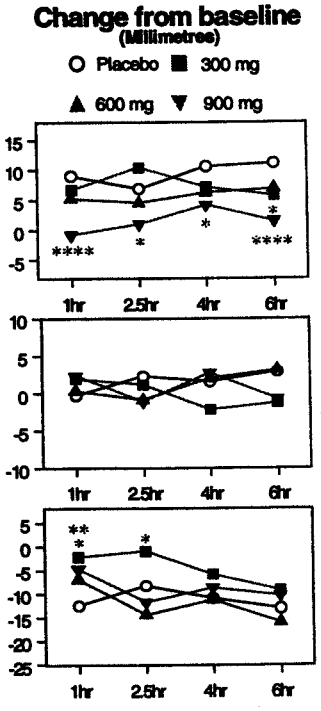


*Figure 10.2. Effects of Melissa officinalis on self-rated mood as measured using Bond-Lader visual analogue scales. The table presents raw scores and change from baseline scores for each dose of M. officinalis (means with standard errors in italics). Graphs represent the change from baseline scores for the three mood dimensions 'alert', 'calm' and 'content' (*, p = 0.05; ***, p = 0.005; ****, p = 0.001; compared to the corresponding placebo score)*

## 10.4. Discussion

The results of the current study suggest that the ingestion of single doses of *Melissa officinalis* can modulate both the mood and cognitive performance of healthy young volunteers in a dose and time dependent manner.

Improvement on the cognitive measures was restricted to the 'Accuracy of Attention' factor, with benefits seen across all time points for the middle dose (600 mg) of *M. officinalis*. However, memory performance was disrupted for all doses of the extract, with relatively clear dose related impairments on the global 'Quality of Memory' measure and the 'Secondary Memory' factor at the 2.5 and 4 hour post-dose testing sessions. Decrements for all doses were also seen on the 'Working Memory' factor, with these being most notable at the earlier testing sessions (1 and 2.5 hours), and for the highest dose of melissa, (900 mg) which evinced reduced performance at all but the penultimate testing sessions..

Mood was also modulated, with significantly increased 'calmness', in comparison to placebo, seen for the highest dose (900 mg) at the first testing session (1 hr), and for the lowest dose (300mg) at both of the first two testing sessions (1 hr and 2.5 hrs). However, self-rated 'alertness' was reduced in comparison to placebo across all testing sessions for the highest dose (900mg). The middle (600 mg) dose was not associated with any significant effects on mood.

The pattern of results can be viewed as largely consistent with both the contemporary use of melissa as a calming agent and mild sedative (Kommission E Monograph, 1984; Bisset and Wichtl 1994), and demonstrations of similar effects in both rodents (Wagner and Sprinkmeyer, 1973; Soulimani *et al,* 1991), and sufferers from severe dementia (Ballard *et al,* 2002). Interestingly the dose associated with the most positive modulation of mood (300 mg), with significantly increased scores on the Bond-Lader 'calm' factor at the two earliest time points, was largely unaffected by the memory decrements associated with the other two doses. This may well suggest, in keeping with the herbalist's maxim that 'less is more', that possible therapeutic doses lie below, or at the lower end of the doses utilised here. Indeed, several

smaller doses of melissa throughout the day may be efficacious in its suggested role in the amelioration of dementia related agitation (Perry *et al,* 1999).

In line with the notion that the lower dose was, on balance, the most beneficial, the middle dose was associated both with cognitive improvements on the 'Accuracy of Attention' factor, and decrements on the memory factors, with no modulation of mood. The highest dose, on the other hand, was detrimental throughout, with the most striking disturbance of memory processes coupled with reduced alertness throughout, and possibly beyond, the six hours that testing encompassed.

Whilst the results here suggest that low doses may be of some utility in the beneficial modulation of mood, and higher doses may well exert a mild sedative effect, there is no evidence to support the historical role for *M. officinalis* in the enhancement of memory, nor the cholinergic properties of the plant (Perry *et al,* 1996, Wake *et al,* 2000). The cognitive effects seen here, albeit for different doses, include positive effects on attention and negative effects on memory, domains which would be expected to be modulated in the same direction in the case of cholinergic action (Feldman *et al,* 1997). It seems unlikely therefore that modulation of this neurotransmitter system underlies the effects seen here, and it is likely, as with all plant extracts, that any effects are as a consequence of several disparate mechanisms. In support of this, reference to the cholinergic binding properties evinced by this extract suggest that nicotinic receptor binding, with an $IC_{50}$ concentration of 11 mg/ml, is much lower than in batches of dried fresh leaf assessed previously, for which $IC_{50}$ values of between 0.08 and 3 mg/ml were obtained (Wake *et al,* 2000). Similarly muscarinic receptor binding, with an $IC_{50}$ concentration of 4 mg/ml, is towards the lower end of the range from the previous study, which elicited $IC_{50}$ values ranging from 0.5 to more than 5 mg/ml (Wake *et al,* 2000). It is possible that these low cholinergic binding properties are the result of a loss of volatile components during the manufacturing process, a possibility that is supported by the inability to detect volatiles

using GCMS. Alternatively they may simply reflect a wide range of receptor binding properties in different batches of the plant.

Whilst the current study does not support a possible role for this specific extract of *M. officinalis* in the amelioration of the cholinergic disturbances associated with Alzheimer's disease (Perry *et al,* 1999), it does not preclude the possibility that an extract, oil or leaf of *M. officinalis* with the previously demonstrated human cortex cholinergic binding properties (Perry *et al,* 1996; Wake *et al,* 2000) may well be efficacious. Indeed a treatment combining both calming effects and beneficial cholinergic modulation may well, given the lack of any known detrimental side effects associated with *M. officianlis*, prove a novel treatment for Alzheimer's disease.

Given this first demonstration in humans of modulation of cognitive performance and mood as a consequence of ingestion of *M. officinalis*, the possibility that a cholinergically active melissa will exert a more favourable profile of cognitive modulation in healthy young volunteers deserves investigation.

# CHAPTER 12. MODULATION OF MOOD AND COGNITIVE PERFORMANCE FOLLOWING ADMINISTRATION OF SINGLE DOSES OF DRIED LEAF OF *MELISSA OFFICINALIS* WITH KNOWN CHOLINERGIC RECEPTOR BINDING PROPERTIES.

## 11.1. Introduction

In Chapter 10 the ingestion of single doses of a commercial *Melissa officinalis* extract was associated with modulation of cognition and mood. The results across the three doses were striking, with largely beneficial effects, restricted to improved mood, at the lowest dose; a mixture of positive effects on attention and negative effects on memory at the middle dose; and largely detrimental effects on both memory performance and mood at the highest dose. This profile of results was unexpected. Specifically, the rationale underlying the study had been that previous *in vitro* studies have demonstrated central nervous system acetylcholine receptor activity, with demonstrations of both nicotinic (Perry *et al.* 1996; Wake *et al.* 2000) and muscarinic (Wake *et al.* 2000) binding properties. It has previously been suggested (Perry *et al,* 1999) that these findings suggest a possible role for *M. officinalis* in the attenuation of the putatively cholinergic cognitive impairments seen in Alzheimer's disease. As such the pattern of results following melissa was expected to be in keeping with cholinergic modulation. The obtained results were, however, more in keeping with contemporary usage of the extract as a calming and mildly sedative agent.

Following completion of the behavioural aspects of the study an *in vitro* investigation of cholinergic receptor binding properties in human brain tissue showed that the extract that had been used had negligible nicotinic, and comparatively low muscarinic binding properties. This leaves open the question of the effects of a *M. officinalis* preparation with cholinergic properties, and the possibility that such a treatment might combine beneficial cholinergic

modulation of cognitive performance with effects on mood that could be conceived of as being potentially beneficial in the treatment of Alzheimer's disease.

With this in mind a number of acquisitions of organic dried *Melissa officinalis* leaf were assessed for *in vitro* human brain tissue cholinergic receptor binding properties. The methodology and results are presented in Appendix I. The current study, therefore, is an investigation of the cognitive and mood effects of single doses of the dried *M. officinalis* leaf found to have the most promising muscarinic and nicotinic binding profile. As such, it uses a similar methodology to the previous chapters with cognitive performance being assessed with the CDR battery and an added 'cholinergically sensitive' Rapid visual information processing task, and mood being assessed with Bond-Lader visual analogue mood scales.

## 11.2. Materials and Methods

### Participants

14 female and 6 male undergraduate volunteers (mean age 19.2 years, age range 18-23) took part in the study which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health, and were taking no illicit social drugs. Additionally they were free of any 'over the counter', herbal, or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Smokers were excluded from the study. All participants abstained from caffeine containing products throughout each study day, and alcohol for a minimum of 12 hours prior to the first testing session of the morning.

### Cognitive Measures

A tailored version of the Cognitive Drug Research (CDR) computerised assessment battery was used. The individual tasks and cognitive measures were as described in detail previously in Chapter 2 (section 2.2. pages 87-93), with the exception that a further task – RVIP - (Rapid visual information processing task – see below) was included in the battery following the choice reaction time task and preceding the spatial memory task. The RVIP task was not included in the original factor analysis (Wesnes *et al,* 2000), and is therefore analysed and reported separately.

*Rapid Visual Information Processing task (RVIP):* This task has been widely used to study the cognitive effects of psychotropic drugs, and has been shown to be sensitive to cholinergic modulation (e.g. Wesnes and Warburton, 1984; Wesnes *et al,* 1990). The participant monitors a continuous series of digits for targets of three consecutive odd or three consecutive even digits.

The digits are presented at the rate of 100 per minute and the participant responds to the detection of a target string by pressing the 'yes' response button as quickly as possible. The task is continuous and lasts for 5 minutes, with 8 correct target strings being presented in each minute. The task is scored for percentage of target strings correctly detected, average reaction time for correct detections, and number of false alarms.

**Subjective mood measure**

Bond-Lader Visual Analogue Scales (Bond and Lader, 1974) were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'.

**Treatments**

On each study day participants received eight capsules that were of identical appearance on each occasion. The individual capsules contained either an inert placebo, or 200 mg of powdered, dried *Melissa officinalis* leaf (Addendum I - Sample A – obtained from Moorbank Botanical Gardens). Depending on the condition to which the participant was allocated on that particular day the combination of capsules corresponded to a dose of either 0 (placebo), 600 mg of *Melissa officinalis* leaf, 1000 mg of *Melissa officinalis* leaf, or 1600 mg of *Melissa officinalis* leaf

**Procedure**

A similar procedure to that in Chapters 2, 3, 4, 7, and 10 was employed. Each participant was required to attend a total of five study days that were conducted seven days apart, to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories with participants visually isolated from each other.

On arrival at their first session on the first day participants were randomly allocated to a treatment regime using a Latin square design which counterbalanced the order of treatments across the four active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered, to allow familiarisation with the test battery and procedure. Data from the foursessions of this practice day were not included in any analysis. Each study day comprised five identical testing sessions. The first was a pre-dose testing session which established baseline performance for that day, and was immediately followed by the day's treatment on days 2 to 5. Further testing sessions began at 1 hour, 3 hours, and 6 hours following consumption of the day's treatment. Each testing session comprised completion of the Bond-Lader visual analogue scales, and the CDR test battery.

**Statistics**

Scores from individual measures were combined to form the 'Quality of Memory' measure, and the five cognitive factor scores. These, the individual task outcome measures, RVIP scores and the three mood outcomes derived from the Bond-Lader visual analogue scales, were analysed as 'change from baseline' using the Minitab statistical package. The initial analysis was made using a two factor (condition x session) Analysis of Variance with repeated measures on both factors. Following the recommendations of Keppel (1991), the omnibus F test was eschewed in favour of planned comparisons, which were made between the placebo and each of the three active treatment conditions (600 mg, 1000 mg and 1600 mg of *Melissa officinalis*) at each time point utilising t tests with the mean squares for 'dose × time × subjects' as an error term. To ensure the overall protection level all testing was two-tailed, comparisons were strictly planned prior to the study, were restricted to the number of conditions minus one at each time-point, and only probabilities associated with these pre-planned comparisons were calculated.

## 11.3. Results

### Baseline scores

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 600 mg, 1000 mg, and 1600 mg *M. officinalis*) for each primary outcome (cognitive factor scores, RVIP scores and mood scale scores) were subjected to a one-way, repeated-measures, ANOVA. There were no significant differences on any measure.

### Individual task outcome measures

Mean pre-dose baseline raw scores, and change from baseline scores for each condition at each post-dose time point on the individual task outcome measures are presented in Table 11.1. Results on individual task outcomes are described in relationship to the overall factor to which they contribute below (memory task results are presented with the relevant factor i.e. 'Secondary' or 'Working' memory).

### Cognitive factor outcome measures

Mean raw baseline scores and change from baseline factor scores for each condition across each session are presented in the table and graphs of Figure 11.1.

#### *Quality of Memory factor*

Planned comparisons of change from baseline data revealed that following the highest dose (1600 mg) of melissa participants performed significantly better than placebo across the memory tasks at both the 3 hour [t(114)=2.35, p = 0.02] and 6 hour hour testing sessions [$t$(114) = 3; p = 0.003]. The same dose also evinced a trend towards improved performance at the 1 hour testing session [$t$(114) = 1.7; p = 0.09]. Performance was also improved in comparison to placebo for the 600 mg condition at the last testing session [t(114)=2.05; p=0.042].

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score | | |
|---|---|---|---|---|---|
| | | | 1 hour | 3 hours | 6 hours |
| Immediate Word Recall (% accuracy) | placebo | 61.50 3.26 | -8.17 3.76 | -10.67 2.89 | -12.33 2.42 |
| | 600mg | 59.00 3.94 | -11.83 3.66 | -7.67 3.91 | -8.67 3.06 |
| | 1000mg | 58.00 3.56 | -9.67 3.00 | -14.50 2.79 | -7.67 3.42 |
| | 1600mg | 59.00 4.98 | -4.00 4.60 | -3.50 3.89* | -4.83 4.48* |
| Simple Reaction time (msecs) | placebo | 253.17 8.16 | 8.34 4.94 | 5.70 4.88 | 4.87 5.15 |
| | 600mg | 265.76 7.44 | -0.77 2.96 | -4.61 4.73 | -7.62 5.37 |
| | 1000mg | 257.75 5.24 | 11.38 9.35 | 6.93 6.24 | 9.02 8.55 |
| | 1600mg | 255.20 8.83 | 15.77 5.81 | 9.01 4.77 | 2.41 6.18 |
| Digit Vigilance Accuracy (%) | placebo | 95.60 0.67 | -0.40 1.02 | -1.10 0.98 | -1.30 0.70 |
| | 600mg | 95.80 0.48 | -0.50 0.66 | -1.30 1.08 | -0.60 0.80 |
| | 1000mg | 94.00 1.15 | 1.80 0.88 | 1.20 1.03 | 1.10 1.11 |
| | 1600mg | 95.60 0.49 | 0.70 0.62 | -0.60 0.74 | -2.40 0.88 |
| Digit Vigilance False alarms (number) | placebo | 0.45 0.15 | -0.20 0.17 | -0.05 0.20 | 0.20 0.26 |
| | 600mg | 0.40 0.13 | 0.40 0.24 | 0.40 0.31 | 0.05 0.18 |
| | 1000mg | 0.40 0.13 | 0.10 0.19 | 0.05 0.21 | 0.05 0.21 |
| | 1600mg | 0.35 0.11 | 0.15 0.18 | -0.05 0.18 | 0.15 0.17 |
| Digit Vigilance Reaction time (msecs) | placebo | 386.38 9.65 | 5.33 7.06 | 4.65 5.78 | -2.28 7.65 |
| | 600mg | 392.64 7.41 | 6.48 3.78 | -2.78 5.01 | -7.04 8.41 |
| | 1000mg | 388.07 8.55 | 7.38 7.52 | 5.64 7.93 | 5.85 6.43 |
| | 1600mg | 395.42 11.19 | 4.78 4.91 | -2.41 5.11 | -3.64 6.01 |
| Choice reaction time accuracy (%) | placebo | 98.67 0.61 | 0.00 0.84 | -0.33 1.02 | -0.33 0.90 |
| | 600mg | 98.33 0.66 | -1.67 1.60 | -1.33 1.04 | -2.00 1.54 |
| | 1000mg | 98.00 0.85 | -2.00 2.06 | 0.33 0.90 | -1.33 1.57 |
| | 1600mg | 97.67 1.39 | 1.00 1.62 | -0.67 1.67 | -0.33 1.71 |
| Choice reactionTime (msecs) | placebo | 390.96 7.99 | 1.18 5.56 | 10.51 8.04 | 6.79 6.91 |
| | 600mg | 393.66 6.68 | 7.91 6.26 | 2.27 8.45 | 8.25 8.77 |
| | 1000mg | 389.17 7.87 | 3.35 7.68 | 15.49 6.74 | 11.89 5.02 |
| | 1600mg | 390.18 10.94 | 16.54 3.79 | 9.78 7.18 | 12.51 7.25 |
| Spatial Memory (%>chance) | placebo | 93.69 1.30 | -4.06 1.73 | -3.88 2.34 | -9.69 4.65 |
| | 600mg | 92.44 1.41 | -2.25 2.90 | -8.69 7.74 | -2.81 1.90 |
| | 1000mg | 90.06 4.14 | 0.06 4.16 | -7.94 7.33 | 0.19 3.91* |
| | 1600mg | 89.88 2.54 | 3.31 2.99 | 3.50 2.16 | 2.13 2.66* |
| Spatial memory Reaction time (msecs) | placebo | 589.36 33.67 | -33.81 21.84 | -46.46 15.30 | -42.71 24.60 |
| | 600mg | 552.49 16.62 | 10.98 14.78 | 18.34 38.82** | -14.38 12.51 |
| | 1000mg | 557.38 21.81 | 20.27 23.32* | 29.15 19.34*** | 19.86 24.94** |
| | 1600mg | 604.91 29.84 | -38.33 24.90 | -43.99 21.12 | -38.86 29.88 |
| NumericWork'g Memory (%>chance) | placebo | 83.67 3.74 | 1.56 1.74 | 1.00 1.48 | -0.55 1.71 |
| | 600mg | 84.45 4.66 | -1.67 1.71 | -0.89 1.66 | -3.22 1.95 |
| | 1000mg | 85.44 3.78 | 0.11 1.38 | -2.44 1.41 | -4.00 1.99 |
| | 1600mg | 86.11 3.83 | -0.34 1.78 | -2.33 1.24 | -3.78 1.75 |
| Numeric Working Memory Reaction Time (msecs) | placebo | 560.25 26.94 | -20.34 11.72 | -35.73 10.80 | -32.86 11.33 |
| | 600mg | 536.07 25.15 | 3.59 9.87 | -5.69 9.95* | -20.25 13.93 |
| | 1000mg | 562.55 28.80 | -22.38 13.49 | -15.76 12.78 | -26.26 15.85 |
| | 1600mg | 558.47 30.92 | -7.85 10.64 | -19.52 16.13 | -38.97 14.74 |
| Delayed Word Recall (% accuracy) | placebo | 41.83 3.96 | -7.17 2.50 | -9.00 3.15 | -14.50 3.46 |
| | 600mg | 39.50 4.27 | -10.33 4.09 | -10.17 3.97 | -13.33 3.97 |
| | 1000mg | 43.33 4.56 | -10.17 3.42 | -13.67 4.43 | -15.00 4.50 |
| | 1600mg | 42.67 4.94 | -7.83 4.41 | -9.17 4.97 | -12.50 4.03 |
| Word Recognition (%>chance) | placebo | 63.67 5.04 | -6.67 5.72 | -13.00 4.65 | -15.90 4.69 |
| | 600mg | 62.33 4.77 | -7.00 5.18 | -7.00 5.06 | -8.00 5.02 |
| | 1000mg | 60.33 4.20 | -9.33 5.06 | -12.00 5.18 | -13.17 4.80 |
| | 1600mg | 59.67 5.20 | -3.00 5.61 | -5.00 6.99 | -10.58 5.88 |
| Word Recognition Reaction time (msecs) | placebo | 709.20 43.12 | -1.46 35.74 | -33.09 24.33 | -80.61 37.63 |
| | 600mg | 690.89 27.81 | 12.35 24.92 | 16.61 23.68 | -48.32 19.32 |
| | 1000mg | 650.11 24.52 | 25.04 21.99 | 49.08 23.34*** | 43.29 28.36***** |
| | 1600mg | 700.00 25.31 | 60.55 32.52* | -2.10 25.99 | -36.14 28.49 |
| Picture Recognition (%>chance) | placebo | 71.50 4.26 | -12.25 3.97 | -11.00 3.53 | -14.50 4.46 |
| | 600mg | 69.25 4.28 | -15.00 5.48 | -9.50 5.03 | -10.00 4.60 |
| | 1000mg | 65.75 5.66 | -1.25 5.31 | -3.25 4.92 | -9.25 5.02 |
| | 1600mg | 68.25 6.09 | -7.00 4.28 | -5.50 5.48 | -6.50 4.25 |
| Picture recognit'n Reaction time (msecs) | placebo | 817.94 42.80 | -34.75 12.67 | -48.51 27.16 | -90.56 34.00 |
| | 600mg | 791.38 35.98 | -25.51 23.83 | -16.77 20.52 | -58.83 26.11 |
| | 1000mg | 779.86 25.17 | 1.62 15.37 | 9.01 35.22* | -30.42 20.65* |
| | 1600mg | 808.49 25.98 | -0.84 24.76 | 6.77 27.92* | -64.30 14.30 |

*Table 11.1. Effects of M. officinalis on individual task outcome measures from the CDR battery,. Mean baseline and change from baseline scores are presented, with standard errors in italics (*, p = 0.05; **, p = 0.01; ***, p = 0.005, *****, = 0.0005 compared to placebo).*

***Secondary Memory Factor***

The 1600 mg dose of melissa was associated, at both the 3 hour [t(114) 2.22; p = 0.029] and 6 hour [t(114) = 2.47; p = 0.015] sessions, with an attenuation of the progressive decline in secondary memory performance seen in the placebo condition. Reference to the single task outcomes showed that performance on the immediate word recall task was improved in comparison to placebo at the 3 hours and 6 hours testing sessions for the 1600 mg dose ([t(114)=2.04; p=0.043] and [t(114)=2.14, p= 0.035] respectively).

***Working Memory Factor***

There were no significant differences on the 'Working Memory' factor. However, reference to the single task outcomes showed that both the 1000 mg and 1600 mg conditions outperformed the placebo condition, in terms of change from baseline, on the spatial memory task at the 6 hour testing session ([*t*(114) = 2.14; p = 0.034] and [*t*(114) = 2.56; p = 0.012] respectively)

***Speed of Memory factor***

All three doses were associated with slowing across the timed memory tasks, in comparison to placebo. The lowest dose (600 mg) evinced a significant slowing at the 3 hour [t(114)=3.4, p=0.001] and 6 hour [t(114)=2.02, p=0.046] testing sessions. The middle dose (1000 mg) led to reduced speed at all time points (1 hour [t(114)=2.2, p=0.03], 3 hours [t(114)=4.53, p=0.00001], and 6 hours [t(114)=4.87, p<0.00001]), and the highest dose showed a similar effect at the 1 hour [t(114)=2, p=0.048]and 3 hour [t(114)=2.02, p=0.046] testing sessions.

Reference to the single task outcomes revealed decrements for all three doses. For the 600 mg dose these took the form of reduced speed of performing both of the working memory tasks at the 3 hour session (spatial memory [t(114)=2.73, p=0.007], numeric working memory [t(114)=2.32, p=0.022]). Similarly, the highest dose (1600 mg) only showed decrements

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 3 hours | 6 hours |
|---|---|---|---|---|---|
| Quality of Memory (%X6) | placebo | 415.86 13.53 | -36.76 11.42 | -46.54 9.25 | -67.47 8.67 |
| | 600mg | 406.97 12.92 | -48.08 9.47 | -43.91 10.19 | -46.03 7.05 |
| | 1000mg | 402.92 18.48 | -30.24 9.78 | -53.80 7.32 | -48.89 13.01 |
| | 1600mg | 405.57 19.79 | -18.86 15.10 | -22.00 15.00 | -36.07 13.83 |
| Secondary Memory (%X4) | placebo | 238.50 11.48 | -34.25 11.06 | -43.67 8.52 | -57.23 9.32 |
| | 600mg | 230.08 10.02 | -44.17 9.10 | -34.33 8.66 | -40.00 6.95 |
| | 1000mg | 227.42 14.48 | -30.42 9.05 | -43.42 8.96 | -45.08 11.86 |
| | 1600mg | 229.58 17.14 | -21.83 13.31 | -23.17 14.50 | -34.42 13.08 |
| Working Memory (%X2) | placebo | 177.36 3.92 | -2.51 2.48 | -2.88 3.06 | -10.24 3.84 |
| | 600mg | 176.88 4.80 | -3.92 3.78 | -9.58 7.56 | -6.03 2.55 |
| | 1000mg | 175.5 5.73 | 0.17 4.60 | -10.38 7.58 | -3.81 4.40 |
| | 1600mg | 175.9 4.70 | 2.98 3.46 | 1.17 2.69 | -1.65 2.71 |
| Speed of Memory (summed msecs) | placebo | 2676.7 127.65 | -90.35 63.09 | -163.8 60.57 | -246.7 88.35 |
| | 600mg | 2570.8 87.29 | 1.41 37.70 | 12.50 49.08 | -141.8 43.50 |
| | 1000mg | 2549.9 80.35 | 24.55 41.43 | 71.48 63.60 | 6.46 59.03 |
| | 1600mg | 2671.8 78.65 | 13.53 52.14 | -58.84 44.95 | -178.3 54.34 |
| Quality of Attention (%) | placebo | 91.90 0.51 | 0.02 0.63 | -0.61 0.65 | -0.95 0.52 |
| | 600mg | 91.88 0.39 | -1.46 0.98 | -1.65 0.79 | -1.32 0.92 |
| | 1000mg | 90.90 0.76 | -0.29 1.12 | 0.66 0.70 | -0.22 1.07 |
| | 1600mg | 91.50 0.75 | 0.66 0.83 | -0.55 0.83 | -1.40 0.67 |
| Speed of Attention (summed msecs) | placebo | 1030.5 21.92 | 14.84 13.78 | 20.87 10.25 | 9.38 14.32 |
| | 600mg | 1052 17.36 | 13.63 9.01 | -5.12 13.52 | -6.41 15.80 |
| | 1000mg | 1035 19.03 | 22.11 16.12 | 28.06 13.75 | 26.76 14.06 |
| | 1600mg | 1040.8 27.27 | 37.09 11.46 | 16.38 11.17 | 11.28 12.17 |

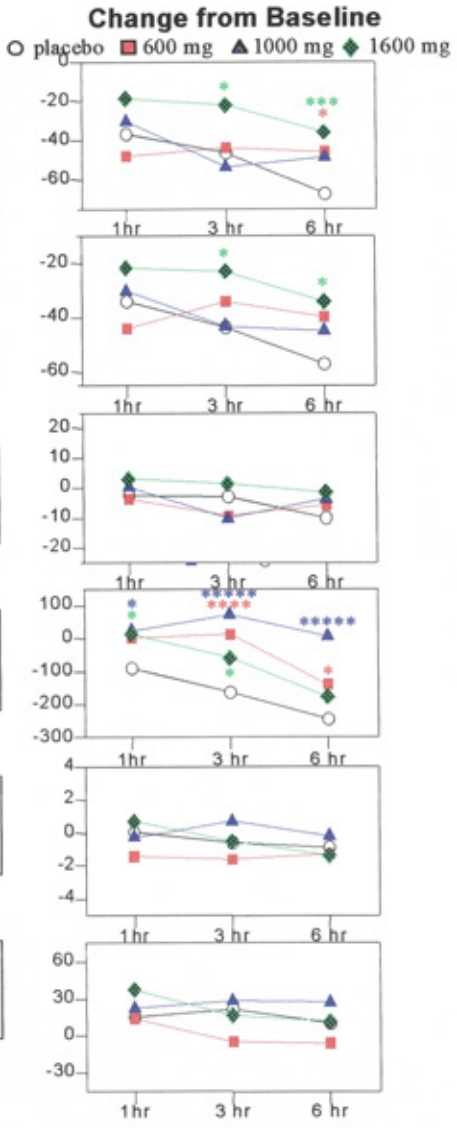


*Figure 11.1. Effects of Melissa officinalis on cognitive measures, 'Quality of Memory', 'Secondary Memory', 'Working Memory', 'Speed of Memory', 'Speed of Attention', and 'Accuracy of Attention'. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of M. officinalis. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; **, p = 0.01, ***, p = 0.005; ****, p = 0.001; *****, p = 0.0005 compared to the corresponding placebo score).Units are as per the table.*

at a single time point on each of the delayed word recognition (1 hour [t(114)=2.3, p=0.023]) and delayed picture recognition tasks (3 hours [t(114)=2. 2, p=0.03]. However, the middle (1000 mg) dose led to comparative decrements at all time points in the speed of performing the spatial memory task (1 hour [t(114)=2.27, p=0.025], 3 hours [t(114)=3.18, p=0.002], and 6 hours [t(114)=2.63, p=0.01]), and at the latter two testing sessions for both the delayed word recognition (3 hours [t(114)=3.04, p=0.003], and 6 hours [t(114)=4.6, p=0.00001] and delayed picture recognition tasks (3 hours [t(114)=2.3, p=0.023], and 6 hours [t(114)=2.41, p=0.018].

***Speed of attention factor***

There were no significant differences on this factor or its component single task outcomes.

***Quality of attention factor***

There were no significant differences on this factor or its component single task outcomes.

**Rapid Visual Information Processing task (RVIP)**

Performance on the RVIP task was also disturbed for both the 600 mg and 1000 mg doses of melissa. The decrements were most marked for the lower dose, with reduced speed of responding, in comparison to placebo, at the 1 and 6 hour testing sessions ([t(114)=2.57, p=0.011] and [t(114)=2.44, p=0.016] respectively). This dose also led to reduced accuracy of performance at the 3 hour testing session [t(114)=2.67, p=0.008] and increased false positive reponses at 1 hour post-dose [t(114)=2.03, p=0.045]. The 1000 mg dose resulted in increased false positive responses at both the 1 hour and 3 hour testing sessions ([t(114)=2.86, p=0.005] and [t(114)=2.4, p=0.018] respectively). There were no decrements following the highest (1600 mg) dose. Baseline and change from baseline data for the RVIP task (% accuracy, response times, and false positives), are presented in the table and graphs of Figure 11.2.

| Measure | | Pre-dose Baseline score | Post-dose change from baseline score 1 hour | 3 hours | 6 hours |
|---|---|---|---|---|---|
| RVIP Accuracy (%) | placebo | 52.25 *5.02* | 2.75 *2.62* | 2.63 *2.99* | -0.13 *3.21* |
| | 600mg | 56.38 *3.95* | -0.13 *2.14* | -3.75 *2.52* | -3.75 *3.36* |
| | 1000mg | 54.38 *4.21* | -0.13 *2.63* | -1.63 *2.10* | -1.13 *2.63* |
| | 1600mg | 54.63 *4.11* | 1.38 *2.44* | 1.00 *1.98* | -1.63 *2.96* |
| RVIP Reaction time (msecs) | placebo | 465.02 *8.86* | -13.80 *7.79* | -13.02 *7.35* | -17.88 *9.69* |
| | 600mg | 464.31 *8.35* | 11.44 *7.31* | -3.62 *9.68* | 6.00 *10.58* |
| | 1000mg | 480.88 *14.13* | -8.83 *8.75* | -20.37 *12.88* | -25.87 *11.33* |
| | 1600mg | 471.30 *12.11* | -8.44 *7.02* | -14.77 *6.22* | -11.91 *10.49* |
| RVIP False positives (number) | placebo | 2.80 *1.08* | -0.55 *0.31* | 0.05 *0.51* | 0.50 *0.44* |
| | 600mg | 2.75 *0.88* | 0.55 *0.38* | 0.15 *0.55* | 0.55 *0.65* |
| | 1000mg | 2.10 *0.85* | 1.00 *0.38*** | 1.35 *0.47** | 0.80 *0.65* |
| | 1600mg | 2.15 *0.58* | 0.40 *0.56* | 0.75 *0.58* | 0.35 *0.32* |

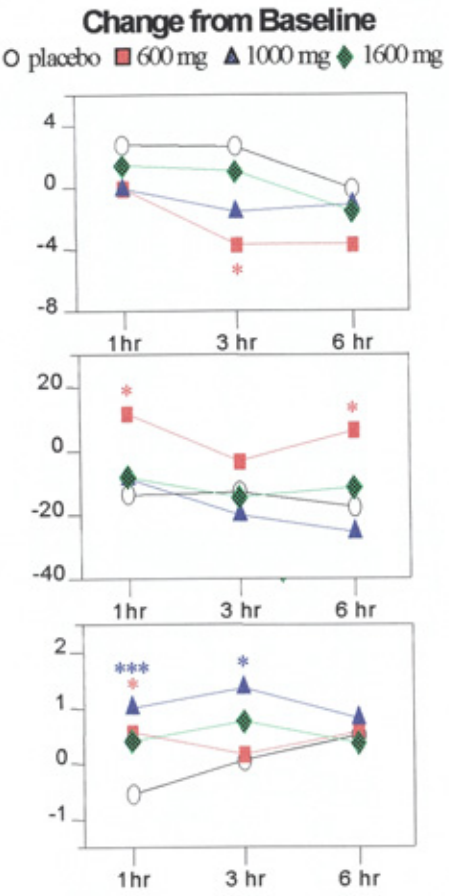


*Figure 11.2 Effects of Melissa officinalis on the accuracy, response times and false positive responses during the five minute rapid visual information processing task. The table presents means (with standard errors in italics) of baseline scores and change from baseline scores for each dose of M. officinalis. Graphs represent the change from baseline scores for the relevant outcome measure (*, p = 0.05; ***, p = 0.005 compared to the corresponding placebo score).Units are as per the table.*

**Subjective mood measures**

*'Alert'* There were no significant differences on the 'alert' factor.

*'Content'* Participants rated themselves as having become less 'content', in comparison to placebo, at the 6 hour testing session following the 1000 mg dose [t(114)=2.4, p=0.018].

*'Calm'* Both 1000 mg and 1600 mg of *M. officinalis* led to increased ratings, in comparison to placebo, on the 'calm' factor derived from the Bond-Lader visual analogue scales. This effect was evident for the 1000 mg dose at 1 hour [t(114)=2.24, p=0.027] and 6 hours[t(114)=1.99, p=0.049], and was sustained throughout the testing day for the 1600 mg dose (1 hour [t(114)=2, p=0.047], 3 hours [t(114)=2.59, p=0.01], and 6 hours [t(114)=2.24, p=0.027]).

The effects of *M. officinalis* on mood are presented in the table and graphs of Figure 11.3.

## 11.4. Discussion

The results of the current study confirm that the ingestion of single doses of *Melissa officinalis* can modulate both the mood and cognitive performance of healthy young volunteers in a dose and time dependent manner.

Improvements were seen in cognitive performance and mood for the highest dose, with improved memory performance and increased 'calmness'. However, decrements were also evident, most notably for the lower doses, in the speed of performing the timed memory tasks. Performance was also disturbed, in terms of both accuracy and speed, on the Rapid Visual Information Processing task.

Whilst performance following all three doses was subject to modulation, the effects became less detrimental as the dose increased. So, the lowest dose saw slowing of both memory tasks and the RVIP, with accuracy also disrupted on the latter task, whilst engendering no mood benefits. The middle dose, on the other hand, showed milder interference on the RVIP and the severest slowing on the memory tasks, but with these effects somewhat offset by improved self-ratings of 'calmness'. The highest dose then, on balance, was largely beneficial, with comparatively modest slowing of memory task performance, but consistent improvements in the accuracy of secondary memory performance, and improved ratings of calmness throughout the post-dose study period.

This pattern of results shows some intriguing similarities, and differences, to those obtained with the commercial extract administered in Chapter 10. The most striking similarity is that the decrements seen within the CDR battery following both treatments are largely confined to the same tasks i.e. the timed memory tasks (specifically the spatial memory, delayed word recognition and delayed picture recognition tasks), with the proviso that the first study saw decrements in terms of accuracy, whilst the current study saw a slowing of performance. Similarly, the dose showing the least decrements on the memory tasks led, as here, to increased

calmness.

The most striking difference then, is that the dose related pattern of results with regards both the negative cognitive and positive mood effects are in the opposite direction in the case of each study. In the first study the lowest dose was the most beneficial, with little impairment of performance and improved 'calmness'. Decrements on the timed memory tasks then increased with the dose, and the highest dose inculcated a negative effect both on mood (reduced alertness) and memory performance. In the current study, on the other hand, the performance decrements on the timed memory tasks (and RVIP) are largely restricted to the lower doses, with improvements in mood increasing with dose.

The most parsimonious explanation for the similarities is that they may reflect the workings of the same mechanism(s). This would suggest that the opposite dose response patterns could reflect the position of each treatment on the same dose response curve. Given the different natures of the extracts, with doses of pure dried leaf in the current study increasing to around about those recommended by herbalists (Bisset and Wichtl, 1994), but a highly concentrated manufactured extract having been used in the previous study, it is highly possible that the actual content of the relevant active constituent/constituents is/are much higher in proportion to weight in the latter. This tentative possibility would suggest that the common effects of all of the doses of both treatments reflect positions on the same inverted U shaped dose response curve of benefits. The doses of dried leaf utilised in the current study could be seen as increasing towards a beneficial window, with the highest dose reflecting entry into a therapeutic dose range, whilst the doses utilised in the former study start in, or slightly above, the beneficial dose range and become generally more detrimental with increased dose.

The apparent contradiction between somewhat impaired speed on memory tasks and improved memory performance for the highest dose (given that this dose's relatively mild impairment on the former, and specific improvement on 'Secondary Memory' tasks argue against this being a 'speed-accuracy' trade off), also suggests that the decrements seen in both studies are not

necessarily memory specific. An explanation that would satisfy Occam's razor is that the tasks involved are simply the most difficult tasks within the battery. Reference to the reaction times for the relevant tasks confirms this, as do the similar decrements that were seen on the non-memory, but equally demanding, RVIP.

If we accept that the decrements in performance are not memory specific, and given the lack of cholinergic receptor binding in the first study, are probably not attributable to direct interaction with nicotinic and muscarinic receptors, then the improved memory performance can be viewed as being attributable to a separate, possibly cholinergic, mechanism. Naturally, given the rationale of the study, it is tempting to suggest that the improved memory performance for the highest dose is directly connected to the demonstrated (Appendix I) high receptor binding properties of the dried leaf used.

Whilst this suggestion is necessarily tentative, the results evinced here do support the suggestion that *M. officinalis* may eventually have a role to play in the treatment of dementia (Perry *et al.* 1996; 1999). Certainly the highest dose utilised here exhibited a profile of cognitive and mood modulation that conforms not only to the historical role of melissa in improving memory, but also to its contemporary usage as a calming agent, and its demonstrated cholinergic receptor binding properties.

Naturally the current study raises a number of questions. The most pertinent is whether increasing the dose of melissa leaf above the maximum used here will confer additional benefits? and how high the dose can be taken before performance and mood become disturbed? Similarly, the question of chronic effects, and specifically, chronic effects in cholinergically challenged populations, deserves to be addressed.

# CHAPTER 12. DISCUSSION

The results of the collection of studies making up this thesis suggest that acute doses of each of the herbal medications under consideration is capable of modulating the cognitive performance of healthy young volunteers. As the specific rationale for investigating the 'Oriental' and 'European' herbs was somewhat different in each case, this discussion will focus on the results of the two series of experiments individually. In each case an initial commentary on the pattern of results for each herbal product and how this relates to the extant literature will be followed by a consideration of possible mechanisms of action.

## 12.1 Oriental herbal extracts

### 12.1.1. Ginkgo biloba

Of the herbal extracts under consideration *Ginkgo biloba* currently attracts the most scientific attention, with research directed both towards its effects in healthy humans and animals, and its efficacy in the treatment of a number of pathological conditions. The studies that examined the effects of ginkgo in the current thesis suggest that acute doses can have a beneficial effect on the cognitive performance of healthy young humans. In Chapter 2 a linear, dose-dependent speeding of the performance of attention tasks was evident from the 2.5 hour testing session until the final testing session at 6 hours post-dose. There was also more modest evidence of improved mnemonic performance with a significant improvement on the global 'Quality of Memory' measure (comprising 'Secondary' and 'Working' memory factors) for the lowest dose (120 mg) at two time points, and a single improvement on the 'Secondary Memory' factor for the middle dose (240 mg) at a single time point. The concurrent serial subtractions experiment (Chapter 5) showed significant improvements in the total number of responses generated for all doses (at 4 hours). The most notable of these being increased responses at both of the later time

points for the 240 mg dose. The subsequent investigation of the effects of the most cognitively beneficial dose from each of the three previous CDR based experiments (Chapters 2, 3, 4), described in Chapter 6, replicated both the enhancement of 'Secondary Memory' performance, and the improved serial subtraction performance, with reduced errors at 4 hours post-dose on the Serial 3s task, and increased responses at 4 and 6 hours post-dose on the Serial 7s task. Mood was also significantly elevated with improvements seen on both the 'alertness' and 'contentedness' Bond-Lader factors across three out of the four testing sessions While the speeded attentional task performance previously seen with the 360 mg dose was not evident, it is noteworthy that this dose had not previously evinced a significant memory enhancing effect and had been less than optimum on the serial subtraction tasks in the earlier studies. Similarly, it was not previously associated with improved mood. As a matter of some interest a recently completed study using the CDR battery and a similar experimental paradigm, but assessing the effects of ginkgo in elderly participants, demonstrated similar improvements as those seen in Chapter 2 on the 'Speed of Attention' factor (Petrini, personal communication). This perhaps suggests that the differing pattern of results across experiments in the current thesis may well reflect either a simple cohort effect, or alternatively a disparity in the verbal instructions (additional to those on-screen) given to participants. A replication of the experiment using standardised verbal instructions to complement the on-screen instructions might elucidate this matter.

Previous demonstrations of directly improved cognitive performance in unimpaired, younger, healthy cohorts following ginkgo include a recent independent groups (n=61) experiment (Stough *et al,* 2001) that purported to demonstrate improvements on a digit span backwards task, speed on a working memory task, and a delayed auditory verbal learning task. It should, however, be noted that these findings are difficult to assess as the paper failed to provide adequate details on doses, tasks employed, or results obtained. Several studies have also investigated the efficacy of ginkgo utilising an acute multiple-dose, placebo-controlled, cross-

over design. Results include improved a demonstration of free picture recall performance (no effect on a numeric working memory task) following the administration of one of two different brands of ginkgo (600 mg) to 12 healthy females (Warot *et al,* 1991). Improved speed of performance on a Sternberg numeric working memory task has also been reported in a small sample of 8 participants administered a single dose of 600 mg of ginkgo (Hindmarch,1986), and during two days of administration of ginkgo (120-300 mg) to 31 adult participants (Rigney *et al,* 1999). The results from the current thesis, with no ginkgo related improvements (Chapters 2 and 6) seen in the speed of performing a Sternberg type numeric working memory task, and with decrements seen in the accuracy of performing this task in both ginkgo studies (Chapter 2 – decrements at 1 and 6 hours: Chapter 6 – decrements at all time points), suggest that these previously demonstrated working memory task improvements can best be ascribed to a simple improvement in performance time rather than the authors' suggestions that their results represent a specific effect on memory processes (Hindmarch, 1986; Rigney *et al,* 1999; Stough *et al,* 2001). Indeed, reference to studies involving chronic administration of ginkgo show consistent evidence of improved performance in terms of either speed of performance, or secondary memory. So, for instance, improvements have been noted in an unimpaired aged cohort (Mix and Crews 2000), and in cognitively impaired aged populations (Allain *et al,* 1993; Oswald *et al,* 1997), on speed of information processing, with improved reaction times *per se* reported in the latter population on a range of tasks (Gessner *et al,* 1985; Rai *et al,* 1991; Schaffler and Reeh, 1985; Wesnes *et al,* 1987a). Consistent improvements have also been seen in the performance of memory and learning tasks (Grassel, 1992; Israel *et al,* 1987), and in scores on cognitive batteries (e.g. SKT, ADAS-COG) that include an assessment of memory performance (e.g. Kanowski *et al,* 1996; Le Bars *et al,* 1997; Maurer *et al,* 1997) following the administration of ginkgo to impaired populations.

The modulation of EEG recordings seen in Chapter 8, with a significant reduction seen in frontal 'eyes closed' theta power in a healthy younger cohort, also falls broadly in line with

evidence of ginkgo having a beneficial effect on theta/alpha waveband ratios (in general reducing theta, while increasing alpha) in the resting 'eyes closed' EEG of cognitively compromised populations (Gessner *et al,* 1985; Hofferberth, 1994; 1995; Itil *et al,* 1998; Kanowski *et al,* 1996; Maurer *et al,* 1997; Pidoux *et al,* 1983; Schulz *et al,* 1991). However, there was no evidence of increased alpha power, as described in younger participants by Itil *et al* (1996). Indeed a decrease in frontal alpha power, with a concomitant increase in occipital alpha power, both of which failed to reach significance, were evident. There was also no evidence of the expected reduced latency of evoked potentials, a phenomena that has been demonstrated in sufferers from age-associated memory impairment (Semlitsch *et al,* 1995), but for which the evidence is somewhat equivocal in healthy young volunteers (Luthinger *et al,* 1995).

### 12.1.2. Panax ginseng

Whilst the pattern of cognitive effects following the ingestion of ginkgo is overwhelmingly positive and reasonably consistent throughout both an extensive literature and the relevant chapters of this thesis, the same cannot be claimed for ginseng. It neither benefits from a substantial methodologically adequate literature with regards its effects in humans, nor has the pattern of results evinced in this thesis been positive in its entirety. In Chapter 3 it was shown that all three doses of ginseng (200, 400 and 600 mg) had a significant positive effect on memory performance and that this enhancement was restricted to secondary memory. These effects were most pronounced for the middle dose (400 mg), with improvement at all time points, and the highest dose (600 mg), with enhancement at three out of the four time points. The lowest dose (200 mg) was only associated with improvement at a single time point. In contrast to these mnemonic improvements the speed of performing attentional tasks was significantly reduced for both the highest (600 mg) and lowest (200 mg) doses of ginseng at the latter two time points, with a single significant reduction in speed of performing the memory

tasks at the 4 hour post-dose testing session for the latter dose. This same dose was also associated both with a drop in self-rated 'alertness', which reached significance by the last testing session (as was the 400 mg dose), and impaired performance on the Serial 7s task across three out of four testing sessions (Chapter 5). The pattern of cognitive effects of the most cognitively advantageous 400 mg dose of ginseng, which had shown marked improvements in secondary memory and no impairment on the 'Speed of Attention' factor in Chapter 3, was replicated in Chapter 6, with a marked modulation of performance, which was again restricted to improved secondary memory performance. This effect on memory processes represents the first reliable demonstration of a modulation of psychological functioning following ginseng.

This dose-dependent pattern of cognitive 'costs' and 'benefits' might appear curious. The middle dose was overwhelmingly positive, and both lower and higher doses associated with less improvement on secondary memory tasks, and impaired speed on attentional tasks. However, this pattern is not entirely inconsistent with demonstrations of the extract's effects on the learning and memory of animals. Whilst ginseng, and various extracts from it, have been shown to have a positive effect on the learning and memory deficits of experimentally brain damaged and ageing rodents (Nitta *et al,* 1995; Wen *et al,* 1996; Zhao and McDaniel, 1998; Zhong *et al,* 2000), similar improvements in normal and young rats tend to be both task and dose dependent. Indeed, multiple-dose studies investigating learning and memory in rats include studies showing inverted-U dose-response relationships (Petkov and Mosharrof, 1987), U-shaped dose response relationships (Petkov *et al,* 1993), improvements restricted to a single dose amongst several (Petkov *et al,* 1993; Petkov and Mosharrof, 1987), and improvements dependent on: dose and administration schedules; the strain of rat; the rat's ability; and the behavioural method employed (Petkov *et al,* 1992). Impairments of performance have also been noted following high doses (Petkov and Mosharrof, 1987), and different fractions (Saito *et al,* 1977; Takagi *et al,* 1972 a,b) of ginseng. The lack of a straightforward effect, seen in both the animal literature and in the experiments described in this thesis, underlines the complexity of ginseng. It's

multiple active components have been shown individually to exert markedly different physiological effects, and the possibility of antagonistic, additive, modulatory and synergistic effects as a consequence of the combination of the multiple components of the whole extract may well explain complex time course and dose-response relationships.

In humans, whilst two studies have concentrated on cognitive functioning (Sorensen and Sonne 1996; D'Angelo *et al,* 1986), and several studies looking at 'quality of life' (Hallstrom *et al,* 1982; Neri *et al,* 1995; Thommessen and Laake, 1996), ergogenic benefits (Forgo and Kirchdorfer, 1981), and blood glucose regulation in diabetics (Sotaniemi *et al,* 1995) have included cognitive tests, no previous studies have assessed either the effects of acute or multiple doses of ginseng (Sotaniemi *et al* used chronic regimes of 100 and 200 mg but collapsed the data from both for their cognitive tests). Improvements in performance in comparison to placebo have been restricted to: improved Randt memory test performance in sufferers from age related memory impairment (Neri *et al,* 1995); improved psychophysical performance in night nurses (Hallstrom *et al,* 1982), and diabetics (Sotaniemi *et al,* 1995); improvements on a mental arithmetic test (D'Angelo *et al,* 1986); and improvements on reaction time tests, restricted to an older sub-section of participants (Forgo and Kirchdorfer, 1981), and to a more difficult auditory simple reaction time test (Sorensen and Sonne, 1996). This last study, with an improvement also seen on the Wisconsin Card Sort Test, is unique in that it evinced improvement on more than one measure from within its typically disparate collections of tasks. It is noteworthy that, in common with an absence of acute or multiple dose studies, to date no studies have employed a comprehensive cognitive assessment battery while assessing the effects of ginseng.

As with direct modulation of cognitive performance, there are few studies, other than an equivocal literature looking at specific ergogenic effects (see: Bahrke and Morgan 1994; 2000), that have looked at the physiological consequences of administration of ginseng to humans. With regards Chapter 8's demonstration of reduced resting blood glucose levels following acute administration of 200 mg of *Panax ginseng* to healthy young participants; one single study

previously investigated the effects of chronic doses of *Panax ginseng* on glucose regulation in non-insulin dependent diabetics. In that study 100 mg and 200 mg/day led to reduced fasted blood glucose levels (Sotaniemi *et al,* 1995). Three studies have also demonstrated blood glucose level reductions during a glucose challenge following large (1-3 g) single doses of *Panax quinquefolium* to both diabetic and non-diabetic cohorts (Vuksan *et al,* 2000a; 2000b; 2001). In Chapter 8 of the current thesis, the absence of any concomitant post-dose modulation of performance on several 'glucose sensitive' tasks makes it difficult to assess the relevance of the lack of a gluco-regulatory effect for doses of ginseng under investigation, although it is tempting to draw parallels between the reduction in blood glucose levels seen following the 200 mg doses of ginseng and decrements in cognitive performance seen in previous chapters. Indeed, impaired performance on the Serial 7s task, as seen in Chapter 5 for the 200 mg dose, would be predicted as a consequence of reduced blood glucose levels (Taylor and Rachman, 1988; Hale *et al,* 1982). Similarly, impaired speed on attentional tasks due to lowered glucose levels could be inferred from observations of increased speed of performance of attentional tasks following augmentation of glucose levels (Benton *et al,* 1994; Owens and Benton, 1994). Where there is a paucity of previous research looking at cognitive and gluco-regulatory effects of ginseng, there is no previous research that has investigated its effects on EEG recordings. In the absence of a comparative literature it seems sufficient to note that the results of Chapter 8 show that the cerebral bio-electrical effects of a single dose of 200 mg of ginseng on the traditionally defined wavebands were similar to, but markedly more pronounced than, those following 360 mg of *Ginkgo biloba.* In both cases significant interactions between treatment and brain region were evident, with significant reductions seen in the frontal power of theta and beta wavebands, and patterns of modulation of the alpha waveband that were similar for both extracts, but which were statistically significant only in the case of ginseng. For ginseng these area specific significant interactions were accompanied by overall treatment related reductions across the scalp in the power of both theta and beta wavebands. The frequency specific effects

of the two treatments, shown in the fractionation of the topographic maps into 0.487 Hz steps, also showed similar 'cool spots' of reduction centred on a number of common frequencies.

Despite the above similarities in waveband modulation it is notable that on other EEG measures the two treatments were markedly dissimilar. Specifically, ginseng also engendered a significant shortening of the latency of the P300 evoked potential component. Whilst a direct relationship between this component of the evoked potential and reaction times on attentional tasks has yet to be established, the finding of reduced latency for ginseng, but not ginkgo, ran somewhat counter to previous observations of the opposite effects of ginkgo and ginseng on the speed of attentional task performance.

While the relevance of the topographic patterns of correlations between changes in cognitive performance and changes in measures of EEG is difficult to assess in the absence of any significant cognitive modulation, the possibility that these effects represent an indication of individual differences in the response to this dose of ginseng is intuitively pleasing. Whether this measure has any utility or not, it seems reasonable to suggest that, in the case of ginseng, further EEG studies, or alternative cerebral activity mapping studies might be appropriate.

### 12.1.3. Ginkgo biloba/Panax ginseng combination

Where the pattern of cognitive effects following single doses of ginkgo and ginseng are somewhat different, the modulation following the 60:100 ginkgo/ginseng combination could be described as possessing some properties of both of these extracts. In Chapter 4 the highest dose of the combination was associated with improved performance at 3 out of 4 testing sessions on the 'Secondary Memory' factor. The lowest (320 mg) and middle (640 mg) doses evinced a lesser mnemonic enhancement, with improvements restricted to single tasks within this factor. As with the less mnemonically active doses of ginseng (Chapter 3), both 320 mg and 640 mg of the combination also resulted in decreased 'Speed of Attention' at the later testing sessions with this effect most notable for the lowest dose. When the most cognitively promising dose

(960 mg) was subsequently taken forward into the direct comparison with a single dose of each of ginkgo and ginseng (Chapter 6) the secondary memory improvement was again replicated. Whilst the results on both of these studies could be described as being markedly similar to ginseng, performance on the serial subtraction tasks was closer to that seen following ingestion of *Ginkgo biloba*. In Chapter 5 the two higher doses of the combination (640 and 960 mg) resulted in fewer errors on the Serial 3s task, and all three doses were associated with improved performance, either in terms of number of responses or errors, on the Serial 7s task. In Chapter 6, following ingestion of the 960 mg dose, participants made more Serial 3 subtractions at the last testing session (6hr), and more subtractions and less errors respectively at the latter two testing sessions (4hr and 6hr) on the Serial 7s task.

It is also noteworthy that, utilising the same tailored version of the CDR assessment battery, and a similar factor structure as used in the current thesis, Wesnes *et al* (1997; 2000) previously showed improvements on the 'Quality of Memory' measure (comprising both 'Secondary' and 'Working' memory factors) following chronic regimens of the ginkgo/ginseng combination administered to 64 Neurasthenic patients (Wesnes *et al*, 1997), and to 256 Healthy middle aged volunteers (Wesnes *et al*, 2000).

### 12.1.4. Mechanisms of action

The results outlined above do not, in themselves, comprise an adequate platform for identifying the mechanisms underlying the cognitive effects of the respective extracts. However, they do suggest a number of speculative possibilities.

***Cholinergic modulation***

It has been suggested that modulation of cholinergic function underlies the cognition enhancing properties of both *Ginkgo biloba* (Nathan, 2000) and ginseng (Lewis *et al*, 1999). Whilst it would be too simplistic to suggest either that any cognitive effects as a consequence of

cholinergic modulation exist without direct interaction with other neurotransmitters, or that the cognitive effects of either ginkgo or ginseng are as a result of modulation of cholinergic function in the absence of any of their other demonstrated neurotransmitter effects, it is still a hypothesis that deserves examination.

In the case of ginkgo a number of converging strands of indirect and direct evidence support the notion of cholinergic modulation. It has been suggested that the cognitive symptoms of Alzheimer's disease are associated, at least in part, with the loss of basal forebrain cholinergic neurons (e.g. Lehericy *et al*, 1989), as indexed by reductions in choline acetyltransferase (e.g. Bierer *et al*, 1995). Evidence from clinical trials suggests that chronic regimens of standardised ginkgo extracts have a modest beneficial effect, comparable to standard anti-cholinesterase treatments (LeBars *et al*, 2000), on the cognitive deficits associated with Alzheimer's disease (e.g. Kanowski *et al*, 1996: LeBars *et al*, 1997; Maurer *et al* 1997). Ginkgo has also been shown to: attenuate learning deficits in rodents in the scopolamine model of cholinergic memory dysfunction (Chen *et al*, 1991; Chopin and Briley, 1992); upregulate hippocampal muscarinic receptor populations (Taylor, 1986); elevate high affinity choline uptake (Kristoikova and Klaschka, 1997); and indirectly modulate acetylcholine release through reversal of age related declines in interacting 5-$HT_{1A}$ receptors (Huguet *et al*, 1994).

With regards ginseng, an *in vitro* cholinergic receptor binding study showed that crude extracts of both *Panax ginseng* and *Panax quinquefolium* exhibited an affinity for the nicotinic receptor in human brain cerebral cortex membranes. *Panax ginseng* also exhibited an affinity, although to a lesser extent, for binding to muscarinic receptors (Lewis *et al*, 1999). Extracts of *Panax ginseng* (Hsieh *et al*, 2000; Jin *et al*, 1999; Nitta *et al*, 1995) and *Panax quinquefolium* (Sloley *et al*, 1999) have also been reported to attenuate scopolamine induced deficits in rodents, with, in the case of the latter study, increased choline uptake demonstrated in synaptosomal preparations. It has also been shown that ginsenoside Rg1 has a direct effect on acetylcholine metabolism in neural tissue, increasing the uptake of choline into nerve endings (Benishin *et al*,

1991; Benishin, 1992), facilitating the release of hippocampal acetylcholine (Benishin *et al*, 1991), increasing the number of cortical choline uptake sites, most particularly in the hippocampus (Benishin, 1992), and increasing choline acetyltransferase levels in rodent brains (Salim *et al*, 1997; Zhang *et al*, 1990), with a similar effect also reported for ginsenoside Rb1 (Zhang *et al*, 1990).

Cholinergic blockade has been shown to preferentially degrade the recall of information from long-term memory, (Drachman and Leavitt, 1974; Peterson, 1977), while sparing working memory, except under conditions of particularly high processing demand (Rusted and Warburton, 1989). It has been proposed that this pattern of deficits reflects disruption of information transfer from short term into long-term storage (Feldman *et al*, 1997; Ghonheim and Mewaldt, 1977). Cholinergic manipulations have also been shown to modulate performance on attentional tasks (Rusted and Warburton, 1989; Wesnes and Warburton, 1983; 1984). Indeed, Blokland (1996) marshals evidence suggesting that memory performance *per se* is not affected by cholinergic activity, but that mnemonic effects are as a consequence of regulation of attentional processes. Similarly, it has been suggested that both the observed memory and attentional effects are as a consequence of cholinergic modulation of central executive functioning (Rusted, 1988; Rusted *et al*, 1991; Rusted and Warburton, 1988; 1991). In any event, in the case of the measures employed in this thesis, previous literature suggests that increased cholinergic activity would be expected to produce a pattern of preferential improvements both in the performance of attentional tasks, and in secondary memory tasks (analogous to longer-term declarative memory), rather than working memory tasks.

The pattern of cognitive modulation following ginkgo falls broadly into this pattern with significant memory improvements restricted to secondary memory tasks (Chapters 2 and 6), and increased attentional performance, in terms of speed on the attentional tasks within the CDR battery (Chapter 2). Speed and/or accuracy on the more complicated mental arithmetic (serial subtraction) tasks, which could be described as relying heavily on central executive resources,

was also improved.

The case for both ginseng and the ginkgo/ginseng combination is less clear-cut, with improvements for both on secondary memory tasks (Chapters 3, 4 and 6), but with deficits in speed on the CDR attentional tasks (Chapters 3 and 4). The combination did, however, evince markedly improved performance on the serial subtraction tasks (Chapters 5 and 6). It is highly unlikely that the attentional 'costs' and memory 'benefits' seen in the CDR based ginseng and combination experiments are both due to cholinergic modulation, and it may be telling that, for both treatments, the two 'cholinergically opposite' effects do not occur in the same doses. Indeed, observations of ginseng related increases in cholinergic parameters specific to hippocampal tissue (Benishin 1992; Benishin *et al*, 1991) indicate that the cholinergic effects of ginseng may be restricted to the memory processes subserved by this structure.

It is also noteworthy that both ginkgo and ginseng modulated a number of EEG measures, with these effects being markedly more pronounced following ginseng. Cholinergic function in key subcortical brain structures is thought to underlie the rhythmicity and synchronisation of cerebral bioelectrical activity (Fisch, 1999; Steriade *et al*, 1993). Whilst the pattern of waveband modulation seen following both extracts does not exactly match the theoretical effects of cholinergic modulation, it is interesting to note that the similarity in the evinced patterns suggests that both extracts may, to some extent, be exerting an influence over the same mechanisms.

***Increased cerebral blood flow and cellular metabolism:***

Interestingly, despite similar attentional task speed deficits as those seen following ginseng, the ginkgo/ginseng combination shares with ginkgo an improvement in performance of the serial subtraction tasks (Chapters 4 and 5). Ginseng, on the other hand, evinces either a dose-specific disruption (Chapter 5) or no effect on performance of the same tasks (Chapter 6). This suggests the possibility that ginkgo is exerting a specific effect that is neither shared nor disrupted by

ginseng.

It has been observed (Nathan, 2000) that the cognition enhancing effects of ginkgo have commonly been attributed to a plethora of neuroprotective and cellular effects resulting from its properties as a platelet-activating factor antagonist and free radical scavenger. These properties also potentially underlie improvements in cerebral cellular metabolism and haemorrheological parameters.

Examples of such improvements, from an extensive literature investigating the effects of ginkgo on hypoxic challenge, include *in vitro* preservation of cellular glucose transport and utilisation (Bruel *et al,* 1989) and mitochondrial respiratory activity (Janssens *et al,* 1999). *In vivo* demonstrations have also shown increased transfer and consumption of cerebral glucose (Rapin *et al, 1986*), and retardation of the breakdown of brain energy metabolism (Oberpichler *et al, 1988*), with, in the case of the latter study, concomitant increases in local cerebral blood flow. In human cohorts, improved blood microcirculation (Chung *et al,* 1999; Jung *et al,* 1990; Koltringer *et al,* 1993), peripheral perfusion (Roncin *et al,* 1996), and haemorrheological fluidity parameters (Jung *et al,* 1990; Koltringer *et al,* 1993; Witte *et al,* 1992) have also been demonstrated. A single study (Kiesewetter *et al,* 1992) also confirmed that the ginkgo/ginseng combination utilised here possessed similar haemorrheological properties in humans as those seen following ginkgo in isolation, with demonstrations of reduced platelet aggregation and increased erythrocyte velocity following single doses.

Ginseng shares with ginkgo a beneficial *in vitro* influence on platelet aggregation (Jung *et al,* 1998; Shi *et al,* 1990), but on the other hand ginseng and its component ginsenosides have also been shown to have opposing effects on a number of parameters including blood pressure (Wood *et al,* 1964), vasoconstriction (Lee *et al,* 1981; Lei and Chiou, 1986), and measures of Hypothalamic-Pituitary-Adrenal axis activity (Luo *et al,* 1993). It is also notable that, in line with previous demonstrations of reductions in circulating blood glucose levels following ingestion of *Panax ginseng* (Sotaniemi *et al,* 1995) and *Panax quinquefolium* (Vuksan *et al*

2000; 2001a; 2001b), the most cognitively deleterious dose of ginseng (200 mg) employed in Chapters 3 and 5 led to a significant reduction in blood glucose levels in healthy young volunteers in Chapter 8.

Given this, it may well be the case that the enhancement of cognition described in this thesis following single doses of ginkgo could be most parsimoniously attributed to simple improved delivery and utilisation of metabolic substrates, whereas this would appear to be unlikely in the case of ginseng. In this respect it is interesting to note not only that Schaffler and Reeh (1985) demonstrated a protection of cognitive performance (choice reaction times) in healthy humans during hypoxic hypoxia, following 14 days treatment with ginkgo, but also that the pattern of cognitive results in this thesis, following administration of ginkgo, bears a number of similarities to those evinced following simple augmentation of both oxygen and glucose levels in healthy young cohorts of humans. As an example, utilising the same battery (CDR) as the current thesis in an investigation of the cognitive effects of oxygen administration, Moss *et al* (1998) also demonstrated dose-dependent improvements on each of the three components of the speed of attention factor (simple, choice, and digit vigilance reaction times), with more restricted improvements observed on the components of the 'Secondary Memory' factor. In line with this, the extensive literature on the cognitive effects of manipulations or fluctuations in blood glucose levels also includes many examples of the attenuation of scopolamine induced cognitive impairments in rodents (e.g. Parsons and Gold, 1992; Stone *et al,* 1991), and also demonstrations in humans of faster reaction times on attentional tasks (Benton *et al,* 1994; Owens and Benton, 1994). Similarly, a fractionation of mnemonic enhancement has been proposed (Foster *et al,* 1998), with clear facilitation of declarative memory performance, and little or no enhancement of working memory tasks. It has been suggested that one prime mechanistic contender underlying such enhancement of attentional and secondary memory tasks is direct increased local provision of acetyl-CoA, and therefore increased acetylcholine synthesis, through the direct oxidative breakdown of glucose (e.g. Gold, 1995, Sunram-Lea and

Foster, 2002; Wenk, 1989), a process that, by its nature, could benefit directly from increased delivery of either or both metabolic substrates.

Cholinergic modulation is only one possible mechanism underlying glucose's facilitatory effects (e.g. Sunram-Lea and Foster, 2002), and it has also been suggested that augmented delivery of glucose may also preferentially enhance performance on cognitively demanding 'fuel limited' tasks. i.e. tasks that are facilitated by the simple delivery of the metabolic substrates necessary to increase localized neuronal activity, and thereby raise the usual ceiling of performance (Kennedy and Scholey 2000; Scholey *et al* 2001; Sunram-Lea *et al,* 2002). In line with this a number of studies have demonstrated greater glucose mediated improvements on difficult, but not easier, versions of a variety of tasks (e.g. Benton *et al,* 1994; Donohoe and Benton, 1999; Donohoe and Benton, 2000; Owens and Benton, 1994), the most pertinent of which here is the Serial 7s subtraction task (Kennedy and Scholey, 2000; Scholey *et al,* 2001) that was also utilised in Chapters 5 and 6. Whilst the results of Chapter 5 showed an improvement restricted to the subjectively easier (Kennedy and Scholey, 2000) Serial 3s subtraction task following ginkgo, the ginkgo/ginseng combination evinced a pattern of significant improvements which was markedly more pronounced for the more difficult Serial 7s task, with improvements in terms of both speed and accuracy, than for the Serial 3s task, on which only accuracy was improved. Similarly the results of Chapter 6 fall into line with the above, with modest improvement seen following the less than optimum doses of ginkgo and the combination on the Serial 3s task (one significant time point improved each), but more pronounced improvements seen on the Serial 7s task (two significant time points each).

This raises the possibility that the cognition enhancing properties of *Ginkgo biloba* are attributable partly to two further mechanisms. Firstly, a simple increased delivery of metabolic substrates, with a subsequent increase in acetylcholine production, leading to a pattern of cognitive improvements in line with the 'cholinergic' pattern outlined in the previous section; and secondly, increased delivery and utilisation of metabolic substrates (both oxygen and

glucose) in active neural tissue during localised demand, leading to improved performance on 'cognitively demanding' tasks.

This putative fractionation of cognitive effects would suggest that the ginkgo/ginseng combination retains properties of both extracts, with the modulatory pattern of ginseng on non-demanding tasks, but with the simple provision of blood-borne metabolic substrates enhancing demanding tasks in a similar manner to ginkgo in isolation.

***Nitric Oxide production***

The pharmacological actions of ginseng are exceedingly complex. Not only do non-ginsenoside components exert pharmacological effects, but the 20 ginsenosides identified to date can individually exert a number of different effects within the same tissue (Attele *et al*, 1999). It has previously been suggested that many of ginseng's physiological effects are as a consequence of enhanced synthesis of nitric oxide (NO) throughout a number of organs and tissues (Gillis, 1997). The range of cells and tissue in which such an effect has been observed include activated macrophages (Fan *et al*, 1995; Friedl *et al*, 2001), peripheral (Chen 1996; Maffei Facino *et al*, 1999; Sung *et al*, 2000), cardiac (Kang *et al*, 1995; Kim *et al*, 1999) and cerebral (Toda *et al*, 2001; Chen *et al*, 1997) vascular tissue, the kidneys (Han and Kim, 1996) muscle tissue (Chen and Lee, 1995; Choi *et al*, 1998; Kim *et al*, 1998; Tamaoki *et al*, 2000) and the brain (Kim *et al*, 1998).

Increased NO synthesis has been proposed, at one time or another, to partially underlie most of the physiological effects of ginseng and ginsenosides, including anti-oxidant and cardio-vascular effects (Gillis, 1997), cardio protection (Maffei Facino *et al*, 1999), neuroprotection (Kim *et al*, 1998), hypothalamic-pitiuitary-adrenal axis nodulation (Kim *et al*, 1998), gluco-regulatory effects (Vuksan *et al*, 2000), and enhancement of immune function (Friedl *et al*, 2001). The enzyme NO synthase has been shown to be present throughout the brain, with a particular prevalence in the cerebellum, and it is reported to be involved in hippocampal long-

term potentiation (Salemme *et al*, 1996). It is therefore tempting to speculate that ginseng also exerts its effects on cognition through the same pathway. In line with this proposition, a number of studies have implicated NO synthesis in the efficacy of several other nootropic treatments (Corasaniti *et al*, 1995; Maurice and Privat, 1997; Reddy and Kulkarni, 1998). A recent review (Prast and Philippu, 2001) presents evidence that NO synthesis is implicated both in general neuronal excitability (including long-term potentiation), and in memory processes. Nitric oxide has also been shown to modulate the release of a number of neurotransmitters, including, but by no means restricted to, serotonin, acetylcholine, and catecholamines. However, the role of NO with regards this modulation of neurotransmission is not entirely straightforward, and whilst increased NO synthesis generally results in increased neurotransmitter release the opposite can also be the case. Similarly, NO is believed to play a dual role in neural transmission, with synaptic actions in the immediate locale of release, and more global actions throughout the brain following release from nitrergic neurons (Prast and Philippu, 2001). Whilst the proposition that NO release underlies the cognitive effects of ginseng is necessarily speculative, the multiplicity of potential effects and modes of action of NO would adequately encompass the less than straightforward pattern of cognitive modulation evinced by ginseng in the current thesis.

It may well also be significant that following ginsenosides the release of NO from endothelial cells has been shown to be specific to the panaxatriol rather than the panaxadiol ginsenosides (Kang *et al*, 1995), whilst memory enhancing effects in rodents are also restricted to extracts with a high, but not a low, ratio of panaxatriol to panaxadiol ginsenoside content (Jin *et al*, 1999).

The mechanisms by which both ginkgo and ginseng act are, as yet, poorly understood. The suggestions above are therefore 'best guesses' based on the profile of results, and the substantial literature dealing with laboratory investigations of possible mechanisms. The

suggestions are by no means exhaustive. Given the complex nature of the multiple active components in both extracts it is highly unlikely that cognitive modulation is as a result either of the action of a single active component, or as a result of action on a single physiological parameter.

### 12.1.5. Oriental Herbs – Conclusion

The relevant chapters of this thesis suggest that the administration of acute doses of both *Ginkgo biloba* and *Panax ginseng,* and indeed their combination, can modulate the mood and cognitive performance of healthy young volunteers.

With regards ginkgo the relevant chapters represent the first acute studies of the effects of *Ginkgo biloba* utilising comprehensive computerised assessment batteries. Ginkgo is attracting an increasing amount of research, including large scale, randomised, controlled trials in a number of patient populations, and the results here are in line with the general tenor of findings throughout this field.

The findings with regards *Panax ginseng* are somewhat more novel. There is little in the way of previous research into the cognitive effects of ginseng, despite the fact that it is the second most commonly taken herbal product (after ginkgo) for memory problems (Hartman Group's Natural Products Census Supplement Report: July 1998 - July 1999). This lack of empirical support for ginseng's effects reflects something of a dichotomy in the respective research approaches to ginkgo and ginseng. *Ginkgo biloba* research was initially driven by the delineation in the 1960's of a high quality, standardised product that facilitated meaningful investigation. The overwhelmingly positive research results have driven the research forward, largely in the West, leading to a substantial, well-balanced literature. Ginseng, on the other hand, is the subject of a far more extensive literature, but the vast majority of it comprises research into the *in vitro*, *ex vivo* and *in vivo* physiological mechanisms that underlie the efficacy of a traditional medical

treatment with assumed efficacy. This research is largely confined to areas of the globe where ginseng is indigenous. The effect of ginseng in humans has largely been investigated in the West, and this small proportion of the overall ginseng literature is markedly equivocal. The nature of the findings with regards ginseng can be attributed both to methodological shortcomings and a lack of standardisation in ginseng products (Bahrke and Morgan, 1994; 2000). On this last point it is notable that the term 'ginseng' covers a huge collection of commercial products, which vary dramatically in overall ginsenoside content. With the exception of research using standardised extracts (G115 being the most commonly used), there is rarely any way of assessing what the experimental treatment actually contains in the way of active ingredients.

By convention, but unsupported by any evidence, there has also been an assumption that ginseng takes some weeks to manifest its effects. The studies in the current thesis are therefore the first to date to look at the effect of single doses of ginseng in humans. The results suggest that *Panax ginseng* exerts more potent acute psychopharmacological effects than *Ginkgo biloba*, and has a specific beneficial effect on secondary memory processes. However, they also suggest that doses other than the optimum can be associated with decrements in non-memory cognitive performance. This does suggest that further research into the effects of ginseng is warranted, and a number of specific questions are raised both by the extant literature and the studies described in this thesis. These include: further clarification of the optimum acute dosage; investigation of the comparative effects of acute and chronic dosage; an elucidation of individual differences in response to ginseng; a comparison of the effects of ginseng extracts varying in the ratio of panaxadiols to panaxatriols; an assessment of the acute effects of ginseng in populations with pathology related memory decrements; and whether the chronic administration of the combination of ginkgo and ginseng confers any additional advantage over and above the single extracts. The above is by no means an exhaustive list of possible

directions of enquiry. However, it is hoped that the results of the relevant chapters in this thesis might encourage further research into some of these questions.

## 12.2. European Herbs

### 12.2.1. Salvia Lavandulaefolia

In Chapter 9 it was shown that each of two doses of essential oil of *S. lavandulaefolia* gave rise to improvements in memory functioning, either in terms of accuracy (25 µl) or speed of performance (25µl and 50µl). Whilst these mnemonic improvements were relatively modest, they were accompanied by an improved rate of responding on both serial subtraction tasks for the 50 µl dose, and on the more demanding Serial 7s task for the 25 µl dose. Mood was also improved for both doses with the lower dose (25 µl) engendering increased 'calmness' on the Bond-Lader scales, while the higher dose (50 µl) improved ratings of 'calmness', 'alertness', and 'contentedness'. With the exception of some evidence of increased errors on the Serial 3s task (possibly due to a regression to the mean following chance underperformance at baseline for the 50 µl dose) the tenor of the results was generally beneficial, with all other significant results suggesting an advantage for the active treatments.

### 12.2.2. Melissa officinalis

The pattern across the two *Melissa officinalis* studies was less straightforward. In the first study (Chapter 10), the most striking result was a pattern of both 'Secondary Memory' and 'Working Memory' decrements, with impairment generally increasing with dosage. The lowest dose (300 mg) was least affected, and also resulted in increased Bond-Lader ratings of 'calmness' at the two earlier testing sessions. While the middle dose under investigation (600 mg) led to more severely disturbed memory performance it also resulted in improved 'Accuracy of Attention' at all time points. For the highest dose (900 mg) the outlook was particularly poor, with the most severe memory decrements, and reduced ratings of 'alertness' at all time points. While these results are not encouraging from the point of view of the rationale of the experiment, they are somewhat in keeping with both the contemporary use of melissa as a calming agent and mild

sedative (Bisset and Wichtl 1994; Kommission E Monograph 1984), and demonstrations of sedative effects in rodents (Soulimani *et al.* 1991; Wagner and Sprinkmeyer, 1973), and calming effects in sufferers from severe dementia (Ballard *et al,* 2002).

In light of *in vitro* results showing negligible cholinergic (particularly nicotinic) receptor binding properties for the extract used in Chapter 10, the second melissa study (Chapter 12) utilised a *M. officinalis* dried leaf that showed particularly promising receptor binding properties (Chapter 11). The results were similar to the first study in that the most striking finding again was a pattern of decrements for all doses that was largely restricted to the same tasks as the previous study (timed memory tasks – spatial memory, delayed word and delayed picture recognition), but which involved decrements in speed rather than accuracy. Again ratings of 'calmness' were increased. One interesting difference between the two studies was that in the second study the dose relationship was reversed, with impairments on the timed memory tasks (and RVIP) reducing with increased dosage, whilst calmness increased. If we suppose that whatever constituent(s) is responsible for these effects survived the manufacturing process that removed the volatiles from the manufactured extract used in the first study relatively intact, it would necessarily be highly concentrated in an extract that is reduced to a fraction of the weight of dried leaves used to produce it. This suggests the possibility that the decreasing decrements with dose on the timed memory tasks following administration of the concentrated manufactured extract, and the increased decrements following the dried leaf, reflect the descending and ascending arms respectively of a U-shaped dose response curve. Similarly, increasing calmness with dose in the first study, and decreasing calmness in the second study may represent the ascending and descending arms of an inverted U-response curve respectively. This possibility also suggests that the highest dose in the second study, utilising dried leaf and therefore providing lower overall dosages of the relevant constituent, might have only just reached the therapeutic window in the *M. officinalis* dose response curves. By the same token, the lowest dose in the first study, using the more concentrated extract, may, have

bordered on or exceeded the beneficial dose range. This still leaves open the question of the added benefits of doses of dried leaf above the 1600 mg utilized here, and doses of the extract below 300 mg.

The second possibility that the pattern of results from the two studies suggest is, given that performance on the difficult RVIP was also disturbed in the second study, that the decrements seen in timed memory tasks across the two studies may well reflect an effect on more difficult timed tasks *per se*, rather than 'memory' tasks. In keeping with this, reference to the reaction times for the tasks that showed the most severe decrements in both studies (spatial memory, delayed word and delayed picture recognition) show that they have the longest latencies. This proposition is also supported by the improvements seen in 'Secondary Memory' performance in the second study for the highest dose of dried leaf. This enhancement cannot be explained in terms of a speed/accuracy trade off, as the dose in question suffered noticeably less slowing on the timed memory tasks, no enhancement of the spatial memory task, and it led to significant improvement on the non-timed immediate word recall task.

If the decrements are simply in the performance of difficult timed tasks then this in itself would suggest that the improvement in memory performance is potentially as a consequence of a different mechanism. Naturally it is tempting to suggest that this mechanism is connected to the substantial CNS cholinergic receptor binding seen for the dried leaf in question.

### 12.2.3. Mechanisms of action

#### *Cholinergic mechanisms*

The rationale for the study of the cognitive and mood effects of both *Salvia lavandulaefolia* and *Melissa officinalis* was provided by the suggestion that, considering the convergence of historical evidence of the use of salvia as a general treatment for the 'brain' or memory across a

number of independent cultures, and in light of research showing pharmacological actions similar to the currently available treatments for Alzheimer's disease, it seems plausible that both herbs may provide novel, well tolerated treatments for dementia related cognitive decline (Mantle *et al,* 2000b; Perry *et al,* 1996; 1998).

The specific mechanisms that it was hypothesised might lead *S. lavandulaefolia* to enhance the cognitive performance of healthy participants was the inhibition of acetylcholinesterase (AChE). Interest in the use of cholinesterase (ChE) inhibitors as treatments for Alzheimer's disease was initially sparked by the observation that plant-derived physostigmine enhanced memory performance in both young and old normal participants (Amenta *et al,* 2001). Currently the only licensed treatments for the disease are the ChE inhibitor Tacrine, and the AChE inhibitors Donepezil, Rivastigmine and Galantamine. In Chapter 9 the *in vitro* assay of the *S. lavandulaefolia* essential oil utilised in this study showed that it exhibited dose dependent inhibition of acetylcholinesterase at similar levels to those found for a *S. lavandulaefolia* oil from the same source (Baldwins & Co, London) in a previous study (Perry *et al,* 2000). Similar AChE inhibition effects for this oil have also previously been demonstrated *ex vivo* (Perry *et al,* 2001) and *in vivo* in selected brain areas of orally administered rats (Perry *et al,* 2002).

The expectation was therefore that behavioural change following sage would fall into the 'cholinergic' pattern described above (pages 284-287). The obtained results showed that cognitive differences, although admittedly not of a great magnitude in comparison to several other studies in this thesis, were largely restricted to improved secondary memory performance, and increased speed on the timed memory tasks. with, on balance, improvement of the serial subtraction tasks. These individual effects can easily be viewed in 'cholinergic' terms, although it should be acknowledged that the central executive (e.g. Rusted *et al,* 1991) and attentional processes (e.g. Blokland, 1996) hypotheses of cholinergic modulation can accommodate a multiplicity of effects. The most dramatic effect of *S. lavandulaefolia* in this instance could be seen as the marked improvement of mood, most specifically for the highest dose, which evinced

improvements on all three factors from the Bond-Lader mood scales. Again, this is not out of keeping with the key role that acetylcholine plays in regulating arousal (Scholey, 2002).

Interestingly, a further sage study (Tildesley *et al,* In Preparation) utilising the same methodology as the study within this thesis, but involving 20 volunteers over 65 years of age, and four separate doses of a different *S. lavandulaefolia* extract, found a markedly stronger memory enhancement across all time points, again restricted to the 'Secondary Memory' factor. The 'Accuracy of Attention' factor was also improved at all time points, and both attention and memory effects were largely restricted to the two lower doses (comparable dosage to the study here) under investigation. Whilst this increase in cognitive benefits above that seen in the sage study in the current thesis may well be due to differences in the extracts used, it is also interesting to note that the 'cholinergic hypothesis' would specifically predict more improvement in a cohort suffering typical age-related down regulation of the cholinergic system.

In the case of *Melissa officinalis* it was hypothesised that the mechanism that had the potential to modulate cognitive function is the ability of active constituents of the plant to bind directly to both nicotinic and muscarinic human brain acetylcholine receptors. This effect has been demonstrated *in vitro* as a consequence of application of extracts from a number of samples of melissa leaf (Perry *et al,* 1996; Wake *et al,* 2000). Muscarinic receptors are relatively preserved in Alzheimer's disease, allowing the possibility that their stimulation could compensate for the loss of nicotinic receptors and cholinergic function (Nordberg, 1992). A number of drugs, including Oxotremorine, Pilocarpin and Arecolene have been proposed as treatments, but side effects, poor oral availability, and short duration of activity, coupled with the complication of the diverse cholinergic effects of the muscarinic receptor subtypes, have led to their abandonment (Amenta *et al,* 2001). Nicotinic stimulation, despite severe reduction in receptor populations, may also be efficacious, and direct administration of nicotine improves cognitive function in Alzheimer's sufferers (Nordberg, 1992) and healthy human populations (Amenta *et*

*al,* 2001). Interestingly, Galantamine, as well as inhibiting AChE, possibly acts as an allosteric modulator of nicotinic receptors, leading to amplification of the effects of ACh (Maelicke, 2000).

In the case of the standardised melissa extract used in Chapter 10 the pattern of cognitive results was unlike that which would be expected from cholinergic modulation, with decrements only on more difficult timed (memory) tasks, and evidence of dose specific improvement on the easier timed tasks. However, the extract was also shown to have little in the way of cholinergic receptor binding properties, suggesting that the cognitive modulation was as a consequence of an unidentified property of the extract. In Chapter 12 the cholinergically active dried leaf identified in Chapter 11 was found to disturb performance of the same tasks as the previous study, but also to engender improved secondary memory performance and 'calmness' at the highest dose. Given that the decrements on both studies can most parsimoniously be explained in terms of a common mechanism it is possible that the enhanced memory performance is as a consequence of cholinergic receptor binding. Given that the *in vitro* assay utilised in this study does not differentiate between the various modes of receptor binding, and also given that the different muscarinic receptor subtypes are known to have markedly differing effects, the cholinergic potential of melissa would benefit from further investigation.

***Other mechanisms***

Whilst the specific rationale for the 'European' herb studies was related to their cholinergic properties it is possible, indeed highly likely in the case of *M. officinalis*, that other properties of the herbs have had some impact on the effects evinced here. In the case of sage, anti-oxidant (Hohmann *et al,* 1999; Mantle *et al,* 2000a), anti-inflammatory, and oestrogenic properties (Perry *et al,* 2001) have also been identified, and both the former and latter of these are known to exert physiological effects that in themselves may modulate cognitive performance (e.g. Ramassamy *et al,* 1992; Shepherd, 2001)

*Melissa offocinalis* shares with *S. lavandulaefolia* free-radical scavenging properties (e.g. Lamaison *et al,* 1993; Tagashira and Ohtake, 1998), but other than that there is a paucity of research investigating its physiological effects. The proposition that the common effects exhibited between the two behavioural studies are unlikely to be mediated by cholinergic receptor binding properties necessarily raises the question as to what mechanism underlies the decrements on the difficult timed tasks in each case. It is interesting to note that in the first study the 600 mg dose of melissa was also associated with improved accuracy on the 'Quality of Attention' factor, comprising the 'underloaded' reaction time tasks (simple and choice reaction time and digit vigilance). One speculative possibility is that both effects could be related to noradrenergic disturbance, which has been shown to preferentially disrupt tasks which require a more difficult sensory discrimination (Aston-Jones, 1985, Berridge *et al.* 1993). The proposition that the results seen here reflect a modulation of noradrenergically mediated vigilance (Aston-Jones, 1985) would account for decrements on the more difficult tasks, whilst allowing the possibility that a noradrenergic reduction in neuronal 'noise' (Aston-Jones *et al,* 1994) might favour the attention to and performance of simple tasks. Noradrenergic modulation may also be implicated in the 'calmness' and 'alertness' effects seen following *M.officinalis.* Again, this possibility, and in particular possible noradrenergic receptor binding properties of *M. officinalis*, would benefit from research.

### 12.2.4. European Herbs - Conclusion

The studies described in Chapters 9, 10 and 12 were specifically driven by the suggestion that both *S. officinalis* and *M. officinalis* might, on the basis of observations of cholinergic modulation, eventually provide novel, cost effective, well tolerated treatments for the cholinergic decrements seen in Alzheimer's disease. These studies were also the first investigation of the cognitive effects of either herb in humans. In the case of *Salvia lavandulaefolia* the results were almost totally beneficial and generally in keeping with the

'cholinergic hypothesis'. The sage study in this thesis has now been followed up with the instigation of a separate research effort.

*Melissa officinalis* is, in its own way, no less promising. Whilst the manufactured extract did not exhibit properties that would recommend its use in treating dementia, the highest dose of 'cholinergic' dried leaf did, and doses in excess of this should be examined. What is particularly interesting about this potential treatment is that the pharmacological arsenal for Alzheimer's disease currently lacks a well-tolerated cholinergic receptor agonist. It is conceivable, in much the same way that sage has been shown to exert more effect in the elderly, that the cholinergic effects of melissa will come into their own in those individuals with a down-regulation of nicotinic receptors. This aside, *M. officinalis* has other properties that might recommend it as a treatment. The first is that advanced Alzheimer's disease commonly involves severe agitation and mood disturbance. In line with the experiments here, and both traditional and contemporary usage of melissa, it seems to have a specific calming or mildly sedative effect. A practical application for this has already been demonstrated in Ballard *et al's* (2002) double-blind, placebo-controlled demonstration of reduced agitation and social withdrawal, and increased time spent in constructive activities in a *M. officinalis* aromatherapy group of sufferers from advanced Alzheimer's disease. The other potential beneficial property of the herb is that, despite a history of usage stretching into millennia, there are no reports of detrimental side effects, even of an anecdotal nature.

## 12.3. Potential methodological limitations

Although the overall pattern of results seen in the current series of studies appears compelling, it is also sensible to acknowledge some possible methodological pitfalls.

The first potential methodological limitation is the nature of the statistical analysis utilised. The general ethos underlying the collection of studies in this thesis is that of a *tabula rasa* approach, concentrating on novel acute dosage experimentation, utilising comprehensive computerised assessment tools. The approach is therefore necessarily exploratory, and as such the statistical analysis of many of the individual studies has been balanced between the need to demonstrate the statistical significance of any findings with a desire not to obscure potentially important findings that can be taken forward into more focussed research. A number of chapters (2, 3, 4, 5, 6, 9, 10, 12) have utilised the same well-used approach to the analysis of multiple dose, multiple time point studies, and have adopted strictly planned comparisons assessing the limited number of questions of true relevance i.e. the effect of each treatment versus placebo at each discrete post-dose time point. The approach adopted is that advocated by Keppel (1991), who states that in the case of experiments specifically designed with a hypothesis in mind *'most researchers conduct analyses relevant to these hypotheses directly without reference to the omnibus F test. (Although the omnibus test may be computed, its significance or non-significance does not modify this particular course of action)'*(p165). In line with this the omnibus Analysis of Variance has been calculated in each case, but reference to it has then been eschewed in favour of planned comparisons using t tests incorporating MS Error. While this is not a contentious approach to analysis it does raise the question of the inflated chance of Type I errors, and on this matter Keppel (1991) notes that *'The most widely used strategy is to evaluate the planned comparisons in the normal way – at the usual PC, or α, level – and to exercise control of the FW rate for post-hoc comparisons through special evaluation procedures designed to cope with the problem '*(p165). In the case of most of the chapters noted above, all of which used the same design and analysis, the pre-planned analysis

is constrained to what Keppel describes as the *'"natural" limit to the number of planned comparisons'* (p167). i.e. the number of conditions minus one, with 3 comparisons (each treatment versus placebo) at each of four time points. (As a point of interest it is worth noting that the three dose/four session design would allow for up to 119 pairwise *post-hoc* comparisons).

On the question of inflated Type I errors Keppel suggests that the number of comparisons that should be made without any kind of correction is arbitrary, depending on the nature of the investigation. He also goes on to propose a modified Bonferroni test that can be used to adjust the probability level that will be taken as significant if desired (in the case of the relevant studies the most conservative interpretation would lead us to set the significance level on any given comparison at p=0.0125), but he also suggests that results that fall between this modified significance and P=0.05 should still be reported, but that judgement should be 'suspended'. In the case of the relevant chapters here the comparisons have been left uncorrected, but interpretation has concentrated on those results that fall into patterns, with notes where relevant in the text urging caution in the over-interpretation of single significant differences. It is noteworthy that whereas the probability of a single comparison being significant by chance alone is 0.05 the hugely decreased probability of two, three or four significant data points appearing for a specific dose, or doses, on a specific measure, are correspondingly far lower than the probability of the type I error which we would wish to protect against.

It is also noteworthy that Keppel also states, again with specific reference to planned comparisons, *'I am in agreement with Davis and Gaito (1984), who argue that an overconcern for Type I error in any particular experiment may actually impede progress in that area of research'* (p178). In the case of the current thesis the investigations include the first study of the acute effects of ginkgo using a computerised battery, and the first studies of the acute cognitive effects of ginseng, a ginkgo/ginseng combination, sage and two distinct types of melissa. As such it is necessary to consider the possibility that there is a relationship between

both dose and time course, and the experiments have been designed accordingly, with several doses and three or four time points. To be over fastidious at this early stage in investigating the effects of these herbs would only serve to obfuscate potentially important findings.

Another area offering potential for confounding the results is the use of change from baseline data. By its very nature this introduces the possibility that baseline differences in performance may exert undue influence over post-dose change from baseline scores, with performance regressing to the mean. In order to reduce this possibility an analysis of pre-dose baseline scores has been undertaken in each case, and caution has been urged in the interpretation of results from the few measures that showed a chance significant difference, or indeed a trend towards a difference, in performance prior to taking the day's treatment.

Whilst the possibility of statistical anomalies exists, it seems unlikely that they have had an undue influence on the results reported here. Indeed, it is particularly noteworthy that all of the most marked cognitive and mood effects of the herbs under investigation have now been replicated, either within the experimentation of the thesis, or alternatively by other research groups using very similar methodology (e.g. Petrini, personal communication; Tildesley *et al*, In Preparation). The obvious exception here is the memory enhancing effect of the 'cholinergic' melissa shown in the last experimental chapter of the thesis, which will hopefully benefit from further research in cognitively compromised populations in the near future.

## 12.4. General Conclusion

The primary aim of this thesis was to examine the cognitive effects of acute doses of a number of herbal products and preparations. Single doses of each of the treatments under investigation have been shown to be effective in modulating cognitive performance and mood, although it is interesting to note that some of the demonstrated effects would not necessarily be described as having been beneficial.

In establishing these effects the use of an integrated computerised assessment battery has proved valuable, and offers some support to suggestions (e.g. Curtis-Prior *et al,* 1999; Vogler *et al,* 1999) that adequately objective and methodologically rigorous research, utilising computer based assessment tools, is a necessity in this field of research. The factor analysis derived cognitive outcomes have also proved useful, and despite the potentially arbitrary labels attached to the factors, have served a valuable heuristic function in the interpretation of the data generated in each of the relevant studies.

One issue that has to be considered when interpreting the studies in this thesis is that of standardisation of herbal products. The systematic study of herbal medications is hindered by a lack of standardisation in a currently unregulated market (Goldman, 2001). As an example, of the treatments utilised here, only *Ginkgo biloba* enjoys anything approaching a satisfactory level of standardisation. The ginkgo extracts prescribed by clinicians and used in research (LI 1270, EGb 761, GK501) all contain invariable levels of the active ingredients. The *Panax ginseng* extract used in this thesis (G115) is itself an extremely highly controlled, standardised extract, with an invariable 4% of ginsenosides. However, the vast majority of the rest of the ginseng products on the market are typified by variable ginsenoside contents and a variety of adulterants (Bahrke and Morgan, 2000; Cui *et al,* 1995; 1996). As such the results evinced in this thesis following G115 are not altogether relevant to the majority of the products on the ginseng market, particularly given the sensitive relationship between cognitive 'costs and benefits' and dosage. While the standardisation of herbal products is a laudable objective, it is

also meaningless without a reasonably full understanding of the active constituents. An interesting illustration of this is provided by the case of St John's Wort, which has seen its market expand exponentially in recent years (Ernst, 2000). This herbal anti-depressant had for a long time been standardised by the content of its assumed active principle, hypericin, whereas evidence now suggests that the actual relevant active component is hyperflorin (Chaterjee *et al*, 1998; Goldman, 2001, Laakman *et al*, 1998). Hyperflorin had only been included in the standardised products, in varying proportions, by dint of the fact that these were whole plant extracts. This lack of knowledge is pertinent both to ginseng, of which the net effect of the combined active constituents is poorly understood, and to the European herbals, for which there is scant research of any kind. It is also interesting to note that the scrupulously standardised *M. officinalis* extract used in Chapter 10 was actually found to have had its volatile components removed by the manufacturing process, and had negligible cholinergic receptor binding properties (although it otherwise still had behavioural effects consistent with its actual common usage). This having been said, in the European herb chapters a concerted effort has been made to quantify the levels of theoretically relevant activity in the treatments used in each instance. Whether the specific assayed activity (AChE inhibition or cholinergic receptor binding) is relevant is open to conjecture, and further study. However, it is notable that the end-point of herbal medication research i.e. the identification and standardised production of treatments with known efficacy, will require extensive knowledge of the constituents and effects of herbal products. Hopefully, the more exploratory chapters in this thesis might generate further such research interest.

It also has to be acknowledged at this point that the collection of studies in this thesis tells us something about the acute psychotropic effects, but little about the chronic effects, of the herbs under investigation. Whilst habituation to the direct pharmacological action of these treatments cannot be ruled out, it seems more likely that the acute effects will remain during extended administration. All four of the plant extracts also have properties that may prove beneficial in

long-term administration. As an example, they all possess appreciable anti-oxidant properties (Mantle *et al*, 2000a). They also individually have attributes [for instance, in the case of *Ginkgo biloba* PAF antagonistic effects (Smith *et al*, 1996), neurotrophic effects for ginsenosides (Salim *et al*, 1997), and oestrogenic properties for *Salvia lavandulaefolia* (Perry *et al*, 2001)], that might lead either to longer term physiological reorganisation of neural tissue and receptor populations, or protection from pathology related damage. It is entirely possible both that chronic benefits will be superimposed on the acute effects demonstrated here, and that the effects of each treatment will be more pronounced in cognitively compromised populations. The methodologically economic, repeated-measures experiments used here will need to be eschewed in favour of larger scale, independent-groups trials in order to elucidate this question adequately.

There is now a general consensus that herbal medications require some form of regulation and standardisation, and that a concerted research effort must be made to identify efficacious treatments (e.g. Ernst, 2000; Goldman, 2001; House of Lords Select Committee on Science and Technology, Sixth Report, 2000). The current thesis was conceived as the initial stage in a '*tabula rasa*' approach to the investigation of the cognitive effects of potentially nootropic herbal treatments. As a starting point a number of computerised assessment tools have been utilised in a series of repeated-measures, acute-dose experiments, designed to include information on the dose-responses and time courses of any cognitive effects evinced. These experiments were conceptualised as providing a first step towards the development of an adequate, wide foundation for the concerted scientific research that will be required to address the substantial question of the efficacy of herbal medications. As such they can be seen as furnishing only limited preliminary information in themselves, but can rather be viewed as raising a number of questions that deserve further investigation.

The stated aims of this thesis were to assess the cognitive effects of administration of single doses of a number of herbal remedies in healthy volunteers. These investigations were to focus

on standardised extracts where these were available, or alternatively, to establish the theoretically relevant *in vitro* properties of those herbal treatments that do not benefit from adequate standardisation. These studies were conceived of as utilising objective, computer-based assessment tools, with specific attention being paid to the time course and dose response relationship of the herbal treatments' effects on cognitive performance. In the case of *Ginkgo biloba* and *Panax ginseng* these studies were also to include investigations of the physiological and/or electrophysiological effects of their administration.

With the exception of the investigation into the effects of single doses of *Ginkgo biloba,* the studies looking at each of these treatments represent either the first investigation of cognitive effects in humans of any nature, or the first investigation of the effects in humans of acute dosage, for that particular plant species. The findings in the experimental chapters of this thesis include:

- Confirmation of enhanced cognitive performance following the acute administration of *Ginkgo biloba.*
- The first demonstrations of cognitive modulation in humans, including a consistent effect on memory performance, following the administration of single doses of both *Panax ginseng,* and a *Panax ginseng/Ginkgo biloba* combination.
- The first direct demonstration of modulation of cerebro-electrical (EEG) activity by the administration of *Panax ginseng.*
- The first demonstration of modulation of blood glucose levels following administration of single doses of *Panax ginseng* to healthy volunteers.
- The first demonstration of modulation of cognitive performance in humans following the administration of *Salvia lavandulaefolia.*
- The first demonstration of modulation of cognitive performance in humans following the administration of both an extract and dried leaf of *Melissa officinalis.*

## References:

Abe K, Cho SI, Kitagawa I, Nishiyama N, Saito H. (1994) Differential effects of ginsenoside Rb1 and malonylginsenoside Rb1 on long term potentiation in the dentate gyrus of rats. *Brain Research.* 649:7-11.

Agrawal A, Pandey MN, Dubey GP. (1993) Management of mental deficiency by an indigeous drug Brahmi (Bacopa monnieri).*Pharmacopsychologia.* 6(1): 1-5.

Agrawal A, Dubey ML, Dubey GP. (1990a) Effects of "mentat" on memory span, attention, galvanic skin resistance (GSR) and muscle action potential (EMG) among normal adults. *Pharmacopsychoecologia.* 3(1): 39-42.

Agrawal A, Dubey ML, Dubey GP. (1990b) Effects of "mentat" on memory and anxiety scores of normal subjects in three age-groups. *Pharmacopsychoecologia.* 3(1):43-45.

Ahlmeyer B, Mowes A, Kriegelstein J, (1999) Inhibition of serum deprivation and staurosporin induced neuronal apoptosis by Ginkgo biloba extract and some of its constituents. *European Journal of Pharmacology.* 367: 423-430.

Aisen PS. (1996) Inflammation and Alzheimer's disease. *Mol. Chem. Neuropathol.* 28:83-88.

Allain H, Raoul P, Lieury A, LeCoz F, Gandon JM, d'Arbigny P. (1993) Effect of two doses of ginkgo biloba extract (EGb 761) on the dual-coding test in elderly subjects. *Clinical Therapeutics.* 15(3): 549-58

Allen JD, McLung J, Nelson AG, Welsch M. (1998) Ginseng supplementation does not enhance healthy young adults' peak aerobic exercise performance. *Journal of the American College of Nutrition.* 17(5):462-6.

Amenta F, Parnetti L, Gallai V, Wallin A. (2001) Treatment of cognitive dysfunction associated with Alzheimer's disease with cholinergic precursors. Inneffective treatments or inappropriate approaches. *Mechanisms of Ageing and Development.* 122: 2025-2040.

Amri H, Ogwuegbu SO, Boujrad N, Drieu K, Papadopoulos V. (1996) In vivo regulation of peripheral-type benzodiazepine receptor and glucocorticoid synthesis by Ginkgo biloba extract EGb 761 and isolated ginkgolides. *Endocrinology*. 1037(12):5707-18

Andrade C, Joseph J, Chandra JS, Vankataraman BV, Rani MA. (1994) ECT-induced anterograde amnesia: can the deficits be minimized? *Convulsive Therapy*. 10(1):59-64..

Aston-Jones G. (1985) Behavioural attributes of locus coeruleus derived from cellular attributes. *Physiological Psychology*. 13: 118-126.

Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T. (1994) Locus coeruleus neurons in monkeys are selectively activated be attended cues in a vigilance task. *Journal of Neuroscience*. 14: 4467-4480.

Attele AS, Wu JA, Yuan C. (1999) Ginseng pharmacology: multiple constituents and multiple actions. *Biochemical Pharmacology*. 58: 1685-1693.

Attella MJ, Hoffman SW, Stasio MJ, Stein. (1989) Ginkgo biloba extract facilitates recovery from penetrating brain injury in adult male rats. *Experimental Neurology*. 105(1):62-71

Avakian EV Jr, Evonuk E. (1979) Effect of Panax ginseng extract on tissue glycogen and adrenal cholesterol depletion during prolonged exercise. *Planta Medica*. 36(1):43-8

Avakian EV, Sugimoto RB, Taguchi S, Horvath SM. (1984) Effect of Panax ginseng extract on energy metabolism during exercise in rats. *Planta Medica*. 50(2):151-4

Bahrke MS, Morgan WP. (1994) Evaluation of the ergogenic properties of ginseng. *Sports Medicine*. 18(4):229-48.

Bahrke MS, Morgan WP. (2000) Evaluation of the ergogenic properties of ginseng: an update. *Sports Medicine*. 298(2):113-133

Bartram, T. (1998). *Bartram's Encyclopedia of Herbal Medicine*. Robinson, London.

Bastianetto S, Zheng WH, Quirion R. (2000) The Ginkgo biloba extract (EGb 761) protects and rescues hippocampal cells against nitric oxide-induced toxicity: involvement of its flavonoid constituents and protein kinase C. *Journal of Neurochemistry*. 74(6):2268-77.

Benishin CG, Lee R, Wang LC, Liu HJ. (1991) Effects of ginsenoside Rb1 on central cholinergic metabolism. *Pharmacology*. 42(4):223-9

Benishin CG. (1992) Actions of ginsenoside Rb1 on choline uptake in central cholinergic nerveendings. *Neurochemistry International*. 21(1):1-5

Benton D, Owens DS, Parker PY. (1994) Blood glucose influences memory and attention in young adults. *Neuropsychologia*. 32: 595-607.

Berridge CW, Arnsten AFT, Foote S. (1993) Noradrenergic modulation of cognitive function: clinical implications of anatomincal, electrophysiological and behavioural studies in animal models. *Psychol Med*. 23: 557-564.

Bhardwaj SK, Srivastava KK. (1995) Effect of a composite Indian herbal preparation, CIHP(III) on avoidance learning during endurance performance of rats. *Indian Journal of Experimental Biology*. 33(8):580-4.

Bhattacharya SK, (1991) Anxiolytic activity of Panax ginseng roots: an experimental study. *Journal of Ethnopharmacology*. 34(1):87-92

Bhattacharya SK. (1994) Nootropic effect of BR-16A (Mentat), a psychotropic herbal formulation, on cognitive deficits induced by prenatal undernutrition, postnatal environmental impoverishment and hypoxia in rats. *Indian Journal of Experimental Biology*. 32(1):31-6.

Bhattacharya A, Ghosal S, Bhattacharya SK. (2001) Anti-oxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *Journal of Ethnopharmacology*. 74(1):1-6.

Bhattacharya SK, Kumar A. (1997) Effect of Trasina, an ayurvedic herbal formulation, on experimental models of Alzheimer's disease and central cholinergic markers in rats. *Journal of Alternative & Complementary Medicine*. 3(4):327-36..

Bhattacharya SK, Kumar A, Jaiswal AK. (1995) Effect of Mentat, a herbal formulation on experimental models of Alzheimer's disease and central cholinergic markers in the rat. *Fitoterapia*. 66: 216-222.

Bierer LM, Haroutunian V, Gabrial S, Knott PJ, Carlin LS, Purohit DP, Perl DP, Schmeider J, Kanof P, Davis KL. (1995) Neurochemical correlates of dementia severity in Alzheimer's disease: relative importance of cholinergic deficits. *Journal of Neurochemistry*. 64: 749-760.

Bisset, N,G., Wichtl,M.,(1994) *Herbal Drugs*. Medpharm. Stuttgart.

Blass JP. (1993) Pathophysiology of the Alzheimers Syndrome. *Neurology*. 43(4) s25-s38.

Blokland A. (1996) Acetylcholine: a neurotransmitter for learning and memory? *Brain Research Reviews*. 21: 285-300.

Bolanos-Jimenez F, Manhaes de Castro R, Sarhan H, Prudhomme N, Drieu K, Fillion G. (1995) Stress-induced 5-HT1A receptor desensitization: protective effects of Ginkgo biloba extract (EGb 761). *Fundamental & Clinical Pharmacology*. 9(2):169-74

Bond, A. and Lader, M. (1974) The use of analogue scales in rating subjective feelings. British Journal of Psychology **47**, 211-218

Brailowsky S, Montiel T . (1997) Motor function in young and aged hemiplegic rats: effects of a Ginkgo biloba extract. *Neurobiology of Aging*. 18(2):219-27

Braquet P, Hosford D.(1991) Ethnopharmacology and the development of natural PAF antagonists as therapeutic agents. *Journal of Ethnopharmacology*. 32(1-3):135-9

Brekhman II, Dardymov IV, (1969) Pharmacological investigation of glycosides from Ginseng and Eleutherococcus. *Lloydia*. 32(1):46-51

British Herbal Pharmacopoeia (1983). British Herbal Medicine Association, Bournemouth.

Brown, D.J. (1996) Phytotherapy - Herbal medicine meets clinical science. *NARD Journal*, May: 41-52

Bruel A, Gardette J, Berrou E, Droy-Lefaix MT, Picard J. (1989) Effects of Ginkgo biloba extract on glucose transport and glycogen synthesis of cultured smooth muscle cells from pig aorta. *Pharmacological Research.* 21(4):421-9

Bruno C, Cuppini R, Sartini S, Cecchini T, Ambrogini P, Bombardelli E.. (1993) Regeneration of motor nerves in bilobalide-treated rats. *Planta Medica.* 59(4):302-7

Calapai G, Crupi A, Firenzuoli F, Marciano MC, Squadrito F, Inferrera G, Parisi A, Rizzo A, Crisafulli C, Fiore A, Caputi AP. (2000) Neuroprotective effects of Ginkgo biloba extract in brain ischemia are mediated by inhibition of nitric oxide synthesis. *Life Sciences.* 67(22):2673-83.

Carnat AP, Carnat A, Fraisse D, Lamaison JL. (1998) The aromatic and Polyphenolic composition of lemon balm (*Melissa Officinalis* L. subsp. Officinalis) tea. *Pharmaceutica Acta Helvetiae* 72: 301-305.

Cerny A, Schmid K (1999) Tolerability and efficacy of valerian/lemon balm in healthy volunteers: a double-blind, placebo-controlled, multicentre study. *Fitoterapia* 70: (3) 221-228

Chatterjee SS, Bhattacharya SK, Wonnemann M, Singer A, Müller WE.(1998) Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sci.* 63:499-510

Chen C, Jin RM, Li Y, Sheng Y, Zhou M, Chen S, Zhou Z. (1991) Improvement of memory in mice by extracts from leaves of Ginkgo biloba L. *China Journal of Chinese Materia Medica.* 16(11):681-3, 704.

Chen X. (1996) Cardiovascular protection by ginsenosides and their nitric oxide releasing action. *Clinical & Experimental Pharmacology & Physiology.* 23(8):728-32

Chen X, Lee TJ. (1995) Ginsenosides-induced nitric oxide-mediated relaxation of the rabbit corpus cavernosum. *British Journal of Pharmacology.* 115(1):15-8.

Chen X, Liu L, Li Z. (1998) Cardiovascular protective effects and NO-mediated cerebrovasorelaxant effects of extract of ginkgo biloba leaves. *Chinese Medical Journal.* 78(9):692-5.

Chen X, Salwinski S, Lee TJ. (1997) Extracts of Ginkgo biloba and ginsenosides exert cerebral vasorelaxation via a nitric oxide pathway. *Clinical & Experimental Pharmacology & Physiology.* 24(12):958-9.

Chermat R, Brochet D, DeFeudis FV, Drieu K. (1997) Interactions of Ginkgo biloba extract (EGb 761), diazepam and ethyl beta-carboline-3-carboxylate on social behavior of the rat. *Pharmacology, Biochemistry & Behavior.* 56(2):333-9.

Cheung F, Siow YL, Oh K. (2001) Inhibition by ginkgolides and bilobalide of the production of nitric oxide in macrophages (THP-1) but not in endothelial cells (HUVEC). *Biochemical Pharmacology.* 61(4):503-10.

Choi SR, Saji H, Iida Y, Magata Y, Yokoyama A. (1996) Ginseng pretreatment protects against transient global cerebral ischemia in the rat: measuement of local cerebral glucose utilization by [14C]deoxyglucose autoradiography. *Biological & Pharmaceutical Bulletin.* 19(4):644-6

Choi YD, Xin ZC, Choi HK. (1998) Effect of Korean red ginseng on the rabbit corpus cavernosal smooth muscle. *International Journal of Impotence Research.* 10(1):37-43.

Chopin P, Briley M. (1992) Effects of four non-cholinergic cognitive enhancers in comparison with tacrine and galanthamine on scopolamine-induced amnesia in rats. *Psychopharmacology.* 106(1):26-30

Christen Y. (2000) Oxidative stress and Alzheimer disease. *American Journal of Clinical Nutrition.* 71(2):621s-629s.

Chung HS, Harris A, Kristinsson JK, Ciulla TA, Kagemann C, Ritch R. (1999) Ginkgo biloba extract increases ocular blood flow velocity. *Journal of Ocular Pharmacology and Therapeutics.* 15(3): 233-40.

Clostre F.(1999) Extrait de Ginkgo biloba (Egb 761). Etat des connaissances a l'aube de l'an 2000. *Ann Pharm Fr.* 57:1S8-1S88.

Cockle SM, Kimber S, Hindmarch I. (2000) The effects of Ginkgo biloba extract LI1370 supplementation on activities of daily living in free living older volunteers: A questionnaire survey. *Human Psychopharmacology.* 15:227-235.

Coghan, T. (1584) *The Haven of Health.* Cited in: Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NSL (1999) Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *Journal of Pharmacy and Pharmacology* 51:527-534.

Corasaniti MT, Paoletti AM, Palma E, Granato T, Navarra M, Nistico G. (1995) Systemic administration of pramiracetam increases nitric oxide synthase activity in the cerebral cortex of the rat. *Functional Neurology.* 10(3):151-5.

Crellin JK, Philpott J (1990) *Herbal Medicine: Past and Present (Vol II),* Duke University Press. London.

Croom, E.M. & Walker, L. (1995) Botanicals in the pharmacy: New life for old remedies. *Drug Topics.* Nov. 6: 84-93

Cui JF. (1995) Identification and quantification of ginsenosides in various commercial ginseng preparations. *Euro J Pharm Sci.* 3(2):77-85.

Cui JF, Garle M, Bjokhem I. (1996) Determination of aglycones of ginsenosides in Ginseng preparations sold in Sweden and in urine samples from Swedish athletes consuming Ginseng. *Scandinavian Journal of Clinical laboratory Investigations.* 56: 151-160.

Curtis-Prior P, Vere D, Fray P. (1999) Therapeutic value of Ginkgo biloba in reducing symptoms of decline in mental function. *Journal of Pharmacy and Pharmacology.* 51:535-541.

D'Angelo L, Grimaldi R, Caravaggi M, Marcoli M, Perucca E, Lecchini S, Frigo GM, Crema A. (1986) A double-blind, placebo-controlled clinical study on the effect of a standardized ginseng extract on psychomotor performance in healthy volunteers. *Journal of Ethnopharmacology.* 16(1):15-22

Dave UP, Chauvan V, Dalvi J. (1993) Evaluation of BR-16 A (Mentat) in cognitive and behavioural dysfunction of mentally retarded children--a placebo-controlled study. *Indian Journal of Pediatrics*. 60(3):423-8.

DeFeo P, Gallai V, Mazzotta G, Crispino G, Torlone E, Perriello G, Ventura M, Santusaneo F, Brunetti P, Bolli GB (1988) Modest decrements in plasma glucose concentrations cause early imnpairment in cognitive function and later activation of glucose counter-regulation in the absence of hypoglaecemic symptoms. *Journal of Clinical Investigation*. 82: 436-444.

De Leo V, Lanzetta D, Cazzavacca R, Morgante G. (1998) Treatment of neurovegatitive menopausal symptoms with a phytotherapeutic agent. *Minerva Ginecol.* 50(5):207-211.

Dhuley JN. (2000) Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. *Journal of Ethnopharmacology*. 70(1):57-63.

Djarmati Z, Jankov RM, DjordjevicA, Ribar B, Lazar D, Engel P. (1992) Carnosic acid 12-Methyl Ether-(-lactone, a Ferruginol type diterpene from Salvia officinalis. *Phytochemistry*. 31(4): 1307-1309.

Donohoe RT. (1997) The relationship between blood glucose and cognitive functioning in young adults. PhD Thesis, University of Wales, Swansea.

Donohoe RT, Benton D. (1999) Cognitive functioning is susceptible to the level of blood glucose. *Psychopharmacology*. 145: 378-385.

Donohoe RT, Benton D. (2000) Glucose tolerance predicts performance on tests of memory and cognition. *Physiology and Behaviour*. 7 (3-4):395-401.

Dorman HPJ, Deans SG, Noble RC. (1995) Evaluation *in vitro* of plant essential oils as natural antioxidants. *Journal of Essential Oil Research*. 7(6):645-651.

Drabaek H, Petersen JR, Winberg N, Hansen KF, Mehlsen J. (1996) The effect of Ginkgo biloba extract in patients with intermittent claudication. *Ugeskr Laeger*. 158(27):3928-31.

Drachman DA, Leavitt J (1974) Human memory and the cholinergic system. *Arch. Neurol.* 30 : 113-121.

Dressing H, Riemann D, Löw H, Schredl M, Reh C, Laux P, Müller WE. (1992) Insomnia: are valerian/balm combinations of equal value to benzodiazepine? *Therapiewoche.* 42; 726-736.

Droy-Lefaix MT. (1997) Effect of the antioxidant action of Ginkgo biloba extract (Egb 761) on aging and oxidative stress. *Age.* 20: 141-149.

Dubey GP, Pathak SR, Gupta BS (1994) Combined effect of Brahmi (Bacopa Monnieri) and Shankhpushpi (convolvulus pluricaulis) on cognitive functions. *Pharmacopsychoecologia.* 7(3): 249-251.

Duverger D, DeFeudis FV, Drieu K. (1995) Effects of repeated treatments with an extract of Ginkgo biloba (EGb 761) on cerebral glucose utilization in the rat: an autoradiographic study. *General Pharmacology.* 26(6):1375-83

Ebert, U., Siepmann, M., Oertel, R., Wesnes, K. and Kirch, W. (1998) Pharmacokinetics and pharmacodynamics of scopolamine after subcutaneous administration. *Journal of Clinical Pharmacology.* **38**: 720-726

Eisenberg, D.M., Davis, R.B., Ettner, S.L. (1998) Trends in Alternative Medicine Use in the United States, 1990-1997: Results of a Follow-up National Survey *The Journal of the American Medical Association.* 280:1569-1575

Engels HJ, Wirth JC. (1997) No ergogenic effects of ginseng (Panax ginseng C.A. Meyer) during graded maximal aerobic exercise. *Journal of the American Dietetic Association.* 97(10):1110-5

Ernst E. (1996) Ginkgo Biloba in treatment of intermittent claudication. A systematic research based on controlled studies in the literature. *Fortschr Med.* 114 (8) 85-7

Ernst E. (2000) Herbal medicines: where is the evidence? Growing evidence of effectiveness is counterbalanced by inadequate regulation. *British medical Journal.* 321 (7258): 395-396.

Ernst E, White A. (2000) 'The BBC Survey of Complementary Medicine Use in the UK'. *Complementary Therapies in Medicine.* 8: 32-36.

Evelyn, J. (1699) *Acetaria.* London (cited in Le Strange 1997).

Fan ZH, Isobe K, Kiuchi K, Nakashima I. (1995) Enhancement of nitric oxide production from activated macrophages by a purified form of ginsenoside (Rg1).*American Journal of Chinese Medicine.* 23(3-4):279-87.

Faruqi S, Andrade C, Ramteke S, Joseph J, Ventkataraman BV, Naga Rani MA (1995) Herbal pharmacotherapy for the attenuation of electroconvulsive shock-induced anterograde and retrograde amnestic deficits. *Convulsive Therapy.* 11(4):241-7.

Feldman RS, Meyer JS, Quenzer LF. (1997) *Principles of Neuropsychopharmacology.* Sinauer. Massachussettes.

Ferrando A, Vila L, Voces JA, Cabral AC, Alvarez AI, Prieto JG. (1999) Effects of ginseng extract on various haematological parameters during aerobic exercise in the rat. *Planta Med.* 65(3):288-90.

Ferrando A, Vila L, Voces JA, Cabral AC, Alvarez AI, Prieto JG (1999) Effects of a standardized Panax ginseng extract on the skeletal muscle of the rat: a comparative study in animals at rest and under exercise. *Planta Med.* 65(3):239-44

Filaretov AA, Bogdanova TS, Podvigina TT, Bodganov AI. (1988) Role of pituitary-adrenocortical system in body adaptation abilities. *Experimental & Clinical Endocrinology.* 92(2):129-36

Fitzl G, Welt K, Schaffranietz L. (1996) Myocardium-protective effects of Ginkgo biloba extract(EGb 761) in old rats against acute isobaric hypoxia. An electron microscopic morphometric study. I. Protection of cardiomyocytes. *Experimental & Toxicologic Pathology.* 48(1):33-9

Folstein M, Folstein SE, McHugh PR. (1975). 'Mini Mental State': A practical method of grading the cognitive state of patients for the clinician. *J Psychiat Resources.* **12:** 189.

Forgo I, Kirchdorfer AM. (1981) On the question of influencing the performance of top sportsmen by means of biologically active substances. *Artl Praxis*. 33(44):1784-9.

Forgo I, Kirchdorfer AM. (1982) The effect of different ginsenoside concentrations on physical work capacity. *Notabene Med.* 12(9) 721-7

Forgo I. (1983) Effect of drugs on physical exertion and the hormonal system of athletes.. *Munchener Medizinische Wochenschrift*. 125(38):822-4

Forgo I, Schimert G. (1985) The duration of effect of the standard ginseng extract G115 in healthy competeitive athletes. *Notabene Med.* 15(9):636-40.

Foster (1996) *Ginkgo: Ginkgo biloba (botanical series 304)* American Botanical Society. Austin. Texas.

Foster JK, Lidder PG, Sunram SI (1998) Glucose and memory: fractionation of enhancement effects. *Psychopharmacology*. 137: 259-270.

Fourtillan JB, Brisson AM, Girault J, Ingrand I, Decourt JP, Drieu K, Jouenne P, Biber A. (1995) Pharmacokinetic properties of Bilobalide and Ginkgolides A and B in healthy subjects after intravenous and oral administration of Ginkgo biloba extract (EGb 761). *Therapie*. 50(2):137-44.

Friedl R, Moeslinger T, Kopp B, Spieckermann PG. (2001) Stimulation of nitric oxide synthesis by the aqueous extract of Panax ginseng root in RAW 264.7 cells. *British Journal of Pharmacology*.134(8):1663-7

Frolich L, Reiderer P. (1995) Free radical mechanisms in dementia of Alzheimer's type and the potential for antioxidative treatment. *Arzneimittelforschung*. 45:443-446.

Fugh-Berman A. (2000) Herb drug interactions. *The Lancet*. 355(9198): 134-138.

Fulder SJ. (1990) *The book of ginseng*.. Healing Arts Press, Rochester.

Gali-Muhtasib H, Hilan C, Khater C. (2000) Traditional uses of *Salvia libanotice* (East meditteranean sage) and the effects of its essential oils. *Journal of Ethnopharmacology.* 71:513-520.

Gasser T, Bacher P, Mocks J.(1982) Transformation towards the normal distribution of broad band spectral parameters of the EEG. *Electroencephalography and Clinical Neurophysiology.* 53: 119-124.

Gessner B, Voelp A, Klasser M. (1985) Study of the long-term action of a Ginkgo biloba extract on vigilance and mental performance as determined by means of quantitative pharmaco-EEG and psychometric measurements. *Arzneimittel-Forschung.* 35(9):1459-65.

Ghonheim MM, Mewaldt SP (1977) Studies on human memory : interactions of diazepam, scopolamine and physostigmine. *Psychopharmacology.* 52 : 1-6.

Gillis CN. (1997) Panax ginseng pharmacology: a nitric oxide link?. *Biochemical Pharmacology.* 4(1):1-8

Gold PE (1995) Role of glucose in regulating the brain and cognition. *International Journal of Clinical Nutrition.* 61: 987-995.

Gold PE, MacLeod KM, Thompson IJ, Frier BM. (1985) Hypoglaecemia induced cognitive dysfunction in diabetes mellitus: effects of hypoglycaemic unawareness. *Physiology and Behaviour.* 58: 501-511

Goldman P. (2001) Herbal Medicines Today and the Roots of Modern Pharmacology. [Academia and Clinic] *Annals of Internal Medicine.* 135: 594-600

Grassel E. (1992) Effect of Ginkgo-biloba extract on mental performance. Double-blind study using computerized measurement conditions in patients with cerebral insufficiency. *Fortschritte der Medizin.* 110(5):73-6

Grieve M. (1931) *A Modern Herbal.* Jonathan Cape (reprinted by Penguin Books Ltd, London, 1980).

Grutzlender J, Morris JC. (2001) Cholinesterase inhibitors for Alzheimer's disease. *Drugs.* 61(1):41-52.

Hale F, Margen S, Rabak D. (1982) Postprandial hypoglycaemia and psychological symptoms. *Biol Psychiat.* **17:** 125-130.

Hallstrom C, Fulder S, Carruthers M. (1982) Effects of ginseng on the performance of nurses on night duty. *Comparat Med East West.* 6(4):277-82.

Han SW. Kim H. (1996) Ginsenosides stimulate endogenous production of nitric oxide in rat kidney.*International Journal of Biochemistry & Cell Biology.* 28(5):573-80.

Hasegawa H, Matsumiya S, Murakami C. (1994) Interactions of ginseng extract, ginseng separated fractions, and some triterpenoid saponins with glucose transporters in sheep erythrocytes. *Planta Med.* 60:153-157.

Hasenohrl RU, Nichau CH, Frisch CH, De Souza Silva MA, Huston JP ,Mattern CM, Hacker R. (1996) Anxiolytic-like effect of combined extracts of Zingiber officinale and Ginkgo biloba in the elevated plus-maze. *Pharmacology, Biochemistry & Behavior.* 53(2):271-5

Hasegawa H, Matsumiya S, Murakami C. (1994) Interactions of ginseng extract, ginseng separated fractions, and some triterpenoid saponins with glucose transporters in sheep erythrocytes. *Planta Med.* 60:153-157.

Hasenohrl RU, Topic B, Frisch C, Hacker R, Mattern CM, Huston JP. (1998) Dissociation between anxiolytic and hypomnestic effects for combined extracts of zingiber officinale and ginkgo biloba, as opposed to diazepam. *Pharmacology, Biochemistry & Behavior.* 59(2):527-35.

Hayman M. (1942) Two minute clinical test for measurement of intellectual impairment in psychiatric disorders. *Arch Neurol Psychiat.* **47:** 454-464.

Hensley K, Aksenova M, Carney JM (1995) Amyloid β-peptide spin trapping: peptide enzyme toxicity is related to free radical spin trap reactivity. *Neuroreport.* 6: 489-492.

Henderson V. (1997) Estrogen, Cognition, and a Woman's Risk of Alzheimer's Disease *American Journal of Medicine*. 103(3A): 11S-18S

Hiai S, Yokohama H, Oura H, Kawashima Y. (1983) Evaluation of corticosterone secretion inducing activities of ginsenosides and their prosapogenins and sapogenins. *Chem Pharm Bull.* 31: 168-174.

Hibatallah J, Carduner C, Poelman MC. (1999) In-vivo and in-vitro assessment of the free-radical-scavenger activity of Ginkgo flavone glycosides at high concentrations. *Journal of Pharmacy and Pharmacology*. 51(12):1435-40.

Hindmarch I. (1986) Activity of Ginkgo biloba extract on short-term memory. *Presse Medicale*. 15(31):1592-4

Hofferberth B (1994) The efficacy of Egb 761 in patients with Senile Dementia of the Alzheimer type, a double blind, placebo controlled study on different levels of investigation. *Human Psychopharmacology*. 9: 215-222.

Hofferberth B. (1995) Influence of *Ginkgo biloba* extract (Egb 761) on neurophysiological and neuropsychological measurements in patients suffering from psychoorganic syndrome. In Christen Y, Courtois Y, Droy-Lefaix MT (Eds.) *Advances in Ginkgo biloba Extraxt Research, Vol 4. Effects of Ginkgo biloba Extract (Egb 761) on Aging and Age-Related Disorders.* Elsevier, Paris.

Hohmann J, Zupko I, Redei D, Csanyi M, Falkay G, Mathe I, Janicsak G. (1999) Protective effects of the aerial parts of *Salvia Officinalis, Melissa Officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. *Planta Medica*. 65: 576-578.

Holmes CS, Koepke KM, Thompson RG, Gyves PW, Weydert JA. (1984) Verbal fluency and naming performance in type I diabetes at different blood glucose concentrations. *Diabetes Care*. 7:454-459.

Hopfenmuller W. (1994) Evidence for a therapeutic effect of Ginkgo biloba special extract. Meta-analysis of 11 clinical studies in patients with cerebrovascular insufficiency in old age. *Arzneimittel-Forschung*. 44(9):1005-13

House of Lords Select Committee on Science and Technology (2000) Sixth Report: Complementary and alternative medicine. HMSO.

Hsieh MT, Peng WH, Wu CR, Wang WH. (2000) The ameliorating effects of the cognitive-enhancing Chinese herbs on scopolamine-induced amnesia in rats. *Phytotherapy Research.* 14(5):375-7..

Hsieh MT, Wu CR, Chen CF. (1997) Gastrodin and p-hydroxybenzyl alcohol facilitate memory consolidation and retrieval, but not acquisition, on the passive avoidance task in rats. *Journal of Ethnopharmacology*. 56(1):45-54.

Hsieh MT, Lin YT, Lin YH, Wu CR. (2000) Radix Angelica Sinensis extracts ameliorate scopolamine- and cycloheximide-induced amnesia, but not p-chloroamphetamine-induced amnesia in rats. *American Journal of Chinese Medicine.* 28(2):263-72.

Hsu HY, Chen YP. (1986) *Oriental Materia Medica: a concise guide.* Oriental Healing Arts Institute. Long Beach, CA.

Huang ZL. (1985) Recent developments in pharmacological study and clinical application of Gastrodia elata in China. *Journal of Modern Developments in Traditional Medicine.* 5(4):251-4.

Huguet F, Drieu K, Piriou A. (1994) Decreased cerebral 5-HT1A receptors during ageing: reversal by Ginkgo biloba extract (EGb 761). *Journal of Pharmacy & Pharmacology.* 46(4):316-8.

Huguet F, Tarrade T. (1992) Alpha 2-adrenoceptor changes during cerebral ageing. The effect of Ginkgo biloba extract. *Journal of Pharmacy & Pharmacology*. 44(1):24-7

Huong NT, Matsumoto K, Watanabe H. (1998) The antistress effect of majonoside-R2, a major saponin component of Vietnamese ginseng: neuronal mechanisms of action. *Methods & Findings in Experimental & Clinical Pharmacology.* 20(1):65-76

Itil TM, Eralp E, Tsambis E, Itil KZ, Stein U. (1996) Central nervous system effects of *Ginkgo biloba*, a plant extract. *American Journal of Therapeutics.* 3: 63-73.

Itil TM, Eralp E, Ahmed I, Kunitz A, Itil KZ. (1998) The pharmacological effects of ginkgo biloba, a plant extract, on the brain of dementia patients in comparison with tacrine. *Psychopharmacology Bulletin.* 34(3):391-7

Jacobs BP, Browner WS (2000) Ginkgo biloba: A living fossil. *American Journal of Medicine.* 108: 341-342.

Jain P, Khanna NK, Trehan N, Pandse VK, Godwhani JL (1994) Antiinflammatory effects of an Ayurvedic preparation, Brahmi Rasayan, in rodents.Indian *Journal of Experimental Biology.* 32(9):633-6.

Janssens D, Michiels C, Delaive E, Eliaers F, Drieu K, Remacle J. (1995) Protection of hypoxia-induced ATP decrease in endothelial cells by ginkgo biloba extract and bilobalide *Biochemical Pharmacology.* 28;50(7):991-9.

Janssens D, Remacle J, Drieu K, Michiels C. (1999) Protection of mitochondrial respiration activity by bilobalide. *Biochemical Pharmacology.* 58(1):109-119.

Jiang KY, Qian ZN. (1995) Effects of Panax notoginseng saponins on posthypoxic cell damage of neurons in vitro. Chung-Kuo Yao Li Hsueh Pao - Acta Pharmacologica Sinica. *16(5):399-402*

Jin SH, Park JK, Nam KY, Park SN, Jung NP. (1999) Korean red ginseng saponins with low ratios of protopanaxadiol and protopanaxatriol saponin improve scopolamine-induced learning disability and spatial working memory in mice. *Journal of Ethnopharmacology.* 66(2):123-9.

Jordan VC. (1998) Designer estrogens. *Scientific American.* 279(4):36-44.

Joseph J, Venkataraman BV, Rani MA, Andrade C. (1994) BR-16A protects against ECS-induced anterograde amnesia. *Biological Psychiatry*. 36(7):478-81.

Jung F, Mrowietz C, Kiesewetter H, Wenzel E. (1990) Effect of Ginkgo biloba on fluidity of blood and peripheral microcirculation in volunteers.*Arzneimittel-Forschung*.40(5):589-93

Jung KY, Kim DS, Oh SR, Lee IS, Lee JJ, Park JD, Kim SI, Lee HK. (1998) Platelet activating factor antagonist activity of ginsenosides. Biological & Pharmaceutical Bulletin.21(1):79-80.

Kang SY, Kim SH, Schini VB, Kim ND. (1995) Dietary ginsenosides improve endothelium-dependent relaxation in the thoracic aorta of hypercholesterolemic rabbit. *General Pharmacology*. 26(3):483-7

Kang SY, Schini-Kerth VB, Kim ND. (1995) Ginsenosides of the protopanaxatriol group cause endothelium-dependent relaxation in the rat aorta. Life Sciences. 56(19):1577-86.

Kanowski S, Herrmann WM, Stephan K, Wierich W, Horr R. (1996) Proof of efficacy of the ginkgo biloba special extract EGb 761 in outpatients suffering from mild to moderate primary degenerative dementia of the Alzheimer type or multi-infarct dementia. *Pharmacopsychiatry*. 29(2):47-56.

Karcher L, Zagermann P, Krieglstein J. *(1984)* Effect of an extract of Ginkgo biloba on rat brain energy metabolism in hypoxia. *Naunyn-Schmiedebergs Archives of Pharmacology. 327(1):31-5.*

Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell, Allen A. (2002) Recent Patterns of Medication Use in the Ambulatory Adult Population of the United States: The Slone Survey. *JAMA*. 287(3):337-344.

Kennedy DO, Scholey AB. 2000. Glucose administration, heart rate and cognitive performance: effects of increasing mental effort. *Psychopharmacology* **149:** 63-71.

Keppel G. (1991) *Design and Analysis*. Prentice Hall. New Jersey.

Khalifa A. (2001) Hypericum perforatum as a nootropic drug: enhancement of retrieval memory of a passive avoidance conditioning paradigm in mice. *Journal of Ethnopharmacology*. 76(1):49-57

Kiesewetter H, Jung F, Mrowietz C, Wenzel E. (1992) Hemorrheological and circulatory effects of Gincosan. *International Journal of Clinical Pharmacology, Therapy, & Toxicology*. 30(3):97-102

Kim DH, Jung JS, Suh HW, Huh SO, Min SK, Son BK, Park JH, Kim ND, Kim YH, Song DK. (1998a) Inhibition of stress-induced plasma corticosterone levels by ginsenosides in mice: involvement of nitric oxide. *Neuroreport*. 9(10):2261-4

Kim HS, Hong YT, Oh KW, Seong YH, Rheu HM, Cho DH, Oh S, Park WK, Jang CG. (1998b) Inhibition by ginsenosides Rb1 and Rg1 of methamphetamine-induced hyperactivity, conditioned place preference and postsynaptic dopamine receptor supersensitivity in mice. *General Pharmacology*. 30(5):783-9

Kim HS, Kang JG, Oh KW (1995a) Inhibition by ginseng total saponin of the development of morphine reverse tolerance and dopamine receptor supersensitivity in mice. *General Pharmacology*. 26: 1071-1076.

Kim HS, Kang JG, Seong YH, Nam KY, Oh KW. (1995b) Blockade by ginseng total saponin of the development of cocaine induced reverse tolerance and dopamine receptor supersensitivity in mice. *Pharmacology and Biochemical Behaviour*. 50:23-27.

Kim HS, Kim K, Oh K. (1999) Ginseng total saponin inhibits nicotine induced hyperactivity and conditioned place preference in mice. *Journal of Ethnopharmacology*. 66:83-90.

Kim HJ, Woo DS, Lee G, Kim JJ. (1998) The relaxation effects of ginseng saponin in rabbit corporal smooth muscle: is it a nitric oxide donor? *British Journal of Urology*. 82(5):744-8.

Kim ND, Kang SY, Park JH, Schini-Kerth VB. (1999) Ginsenoside Rg3 mediates endothelium-dependent relaxation in response to ginsenosides in rat aorta: role of K+ channels.*European Journal of Pharmacology*. 367(1):41-9.

Kim YC, Kim SR, Markelonis GJ, Oh TH. (1998) Ginsenosides Rb1 and Rg3 protect cultured rat cortical cells from glutamate-induced neurodegeneration. *Journal of Neuroscience Research.* 53(4):426-32.

Kimura M; Waki I; Chujo T; Kikuchi T; Hiyama C; Yamazaki K; Tanaka O. (1981) Effects of hypoglycemic components in ginseng radix on blood insulin level in alloxan diabetic mice and on insulin release from perfused rat pancreas. *Journal of Pharmacobio-Dynamics.* 4(6):410-7.

Kimura I. Nakashima N. Sugihara Y. Fu-jun C. Kimura M. (1999) The antihyperglycaemic blend effect of traditional chinese medicine byakko-ka-ninjin-to on alloxan and diabetic KK-CA(y) mice. *Phytotherapy Research.* 13(6):484-8.

Kimura Y, Okuda H, Arichi S. (1988) Effects of various ginseng saponins on 5-hydroxytryptamine release and aggregation in human platelets. *Journal of Pharmacy & Pharmacology.* 40(12):838-43

Klein J, Chatterjee SS, Loffelholz K. (1997) Phospholipid breakdown and choline release under hypoxic conditions:inhibition by bilobalide, a constituent of Ginkgo biloba. *Brain Research.* 755(2):347-50

Kleijnen J, Knipschild P. (1992a) Ginkgo biloba. *Lancet.* 12;340(8833):1474

Kleinen J, Knipschild P. (1992b) Ginkgo Biloba for cerebral insufficiency. *Br J Clin Pharm.* 34: 352-358

Knapik JJ, Wright JE, Welch MJ. (1983) The influence of Panax Ginseng on indices of substrate utilisation during repeated exhaustive exercise in man. *Fed Proc.* 42:336.

Knapp MJ, Knopman DS, Solomon PR, Pendelbury WW, Davis CS, Gracon SI (1994) A 30 week randomised controlled trial of high dose tacrine in patients with Alzheimer's disease. *JAMA.* 271: 985-991.

Kobuchi H, Droy-Lefaix MT, Christen Y, Packer L. (1997) Ginkgo biloba extract (EGb 761): inhibitory effect on nitric oxide production in the macrophage cell line RAW 264.7.*Biochemical Pharmacology.* 53(6):897-903.

Koch-Heitzmann, I. Schultze, W. (1988) 2000 Jahre Melissa officinalis; *Z Phytother.* 9; 77-85

Koltringer P, Langsteger W, Klima G, Reisecker F, Eber. (1993) Hemorrheologic effects of ginkgo biloba extract EGb 761. Dose-dependent effect of EGb 761 on microcirculation and viscoelasticity of blood. *Fortschritte der Medizin.* 111(10):170-2

Kommission E Monograph (1984) Melissenblàtter; Bundesanzeiger, 05.12.

Krauskopf R, Guinot P, Peetz HG (1983) Long term on line analyses demonstrating the pharmaco-dynamic effect of a defined Ginkgo Biloba extract. *Beaufour-Schwabe Internat Report,* 1983.

Krieglstein J, Beck T, Seibert A. (1986) Influence of an extract of Ginkgo biloba on cerebral blood flow and metabolism. Life Sciences. 39(24):2327-34

Kristoikova Z, Klaschka J. (1997) In vitro effect of Ginkgo biloba extract (EGb 761) on the activity of presynaptic cholinergic nerve terminals in rat hippocampus. *Dementia & Geriatric Cognitive Disorders.* 8(1):43-8

Kudo K, Tachikawa E, Kashimoto T, Takahashi E (1997) Comparison between ginsenoside Rg2 and Rg3 effects on catecholamine secretion from bovine adrenal chromaffin cells. *Japanese Journal of Pharmacology.* 73:79.

Kulkarni SK, Verma A (1992) Evidence for nootropic effect of BR-16A (Mentat), a herbal psychotropic preparation, in mice. *Indian Journal of Physiology & Pharmacology.* 36(1):29-34.

Kunkel H (1993) EEG profile of three different extractions of *Ginkgo biloba. Neuropsychobiology.* 27(1):40-48.

Kuo SC, Teng CM, Lee JC, Ko FN, Chen SC, Wu TS. (1990) Antiplatelet components in Panax ginseng. *Planta Medica.* 56(2):164-7.

Laakmann G, Dienel A, Kieser M. (1998) Clinical significance of hyperforin for the efficacy of Hypericum extracts on depressive disorders of different severities. *Phytomedicine.* 5:435-42

Lamaison JL, Petitjean-Freytet C, Carnat A. (1991) Medicinal Lamiaceae with antioxidant properties, a potential source of rosmarinic acid. *Pharmaceutica Acta Helvetiae. 66(7):185-8,*.

LeBars PL, Kieser M, Itil K. (2000) A 26 week analysis of a double blind placebo controlled trial of the Ginkgo biloba extract Egb 761 in dementia. *Dementia and Geriatric Cognitive Disorders.* 11: 230-237.

Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. (1997) A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American EGb Study Group *JAMA.* 278(16):1327-32.

Lehericy S, Hirsch EC, Cervera P, Hersh LB, Hauw J, Ruberg M, Agid Y. (1989) Selective loss of cholinergic neurons in the ventral striatum of patients with Alzheimer's disease. *Proceedings of the National Academy of Science.* 86:8530-8584.

Lee MO, Kim CY, Clifford DH. (1981) Effect of ether, ethanol and aqueous extracts of ginseng on cardiovascular function in dogs. *Canadian Journal of Comparative Medicine.* 45(2):182-7

Lei XL, Chiou GC. (1986) Cardiovascular pharmacology of Panax notoginseng (Burk) F.H. Chen and Salvia miltiorrhiza. *Am J Chin Med.* 14(3-4):145-52

Le Poncin Lafitte M. Rapin J. Rapin JR. (1980) Effects of Ginkgo Biloba on changes induced by quantitative cerebral microembolization in rats. *Archives Internationales de Pharmacodynamie et de Therapie.* 243(2):236-44.

Le Strange, R. (1997) *A History of Herbal Plants.* Morrison & Gibb, London

Leung, A.Y. and Foster S. (1996). *Encyclopedia of Common Natural Ingredients.* John Wiley, Chichester.

Lewis WH, Zenger VE, Lynch RG. (1983) No adaptogen response of mice to ginseng and Eleutherococcus infusions. *Journal of Ethnopharmacology.* 8(2):209-14

Lewis, R., Wake, G., Court, G., Court, J.A., Pickering, A.T., Kim, Y.C. and Perry, E.K. (1999) Non-ginsenoside nicotinic activity in *Ginseng* species. *Phytotherapy Research.* 13:59-64

Li Z, Nakaya Y, Niwa Y, Chen X. (2001) K(Ca) channel-opening activity of Ginkgo Biloba extracts and ginsenosides in cultured endothelial cells. *Clinical & Experimental Pharmacology & Physiology.* 28(5-6):441-5.

Liberti LE, Der Manderosian A. (1978) Evaluation of commercial ginseng products. *Journal of Pharmaceutical Science. 67* (10): 1487-9.

Lim JH, Wen TC, Matsuda S, Tanaka J, Maeda N, Peng H, Aburaya J, Ishihara K, Sakanaka M. (1997) Protection of ischemic hippocampal neurons by ginsenoside Rb1, a main ingredient of ginseng root. *Neurosci Res.* 28(3):191-200.

Liu CX, Xiao PG.(1992) Recent advances on ginseng research in China. *J Ethnopharmacol* 36:27-38.

Liu M, Zhang JT. (1995) Protective effects of ginsenoside Rb1 and Rg1 on cultured hippocampal neurons. *Yao Hsueh Hsueh Pao - Acta Pharmaceutica Sinica.*30(9):674

Logani S, Chen MC, Tran T, Le T, Raffa RB (2000) Actions of Ginkgo Biloba related to the potential utility for the treatment of conditions involving cerebral hypoxia. *Life Sciences* 67:1389-1396.

Luo YM, Cheng XJ, Yuan WX. (1993) Effects of ginseng root saponins and ginsenoside Rb1 on immunity in cold water swim stress mice and rats. *Chung Kuo Yao Li Hsueh Pao.* 14(5):401-4

Luthinger R, d'Arbigny P, Macher JP. (1995) *Ginkgo biloba* extract (Egb 761), EEG and event related potentials mapping profile. *In Christen Y, Courtois Y, Droy-Lefaix MT (Eds)* Advances in *Ginkgo biloba* extract research, vol 4. Effects of *Ginkgo biloba* extract (Egb 761) on Aging and Age-related disorders. Elsevier. Paris.

Lyubimov II, Borzenkov VM, Chepurnova NE, Chepurnov SA. (1997) Effect of a polysaccharide fraction of ginseng root on learning and memory in rats (using an active escape response as an example). *Neuroscience & Behavioral Physiology*. 27(5):555-8

MacKintyre A. (1996) *The Complete Floral Healer*. Gaia Books Ltd. London.

Maelicke A. (2000) Allosteric modulation of nicotinic receptors as a treatment strategy for Alzheimer's disease. *Dement Geriatr Cogn Disord.* 11(1):11-8

Maffei-Facino R, Carini M, Aldini G, Berti , Rossoni G. (1999) Panax ginseng administration in the rat prevents myocardial ischemia-reperfusion damage induced by hyperbaric oxygen: evidence for an antioxidant intervention. *Planta Medica*. 65(7):614-9.

Major RT. (1967) The ginkgo, the most ancient living tree. *Science* 15;157(794):1270-3

Mantle D, Eddeb F, Pickering A (2000a) Comparison of relative antioxidant activities of British medicinal plant species in vitro. *Journal of Ethnopharmacology*. 72:47-51.

Mantle D, Pickering AT, Perry EK (2000b) Medcinal plant extracts for the treatment of dementia. A review of their pharmacology, efficacy and tolerability. *CNS drugs*. 13 (3):201-213.

Marasco A, Vargas Ruiz R,Salas Villagomez A, Begona Infante C. (1996) Double-blind study of a multivitamin complex supplemented with ginseng extract. *Drugs Under Experimental & Clinical Research.* 22(6):323-9

Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. (1994) The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochemical & Biophysical Research Communications*. 201(2):748-55

Markesbery WR. (1997) Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biology and Medicine*. 23:134-147.

Martinez B, Staba EJ. (1984) The physiological effects of Aralia, Panax and Eleutherococcus on exercised rats. *Japanese Journal of Pharmacology*. 35(2):79-85

Mathews DR, Holman RR, Brown E, Steenson J, Watson A, Hughes S, Scott D. (1987) Pen sized digital 30s blood glucose meter. *Lancet*. 1:778-779.

Matsuda H, Samukawa K, Kubo M. (1990) Anti-inflammatory activity of ginsenoside Ro. *Planta Medica*. 56:19-23.

Matsuda H, Samukawa K, Kubo M. (1991) Anti-hepatitic activity of ginsenoside Ro. *Planta Medica*. 57:523-526.

Maurer K, Ihl R, Dierks T, Frolich L. (1997) Clinical efficacy of Ginkgo biloba special extract EGb 761 in dementia of the Alzheimer type. *J Psychiatr Res*. 31(6):645-55.

Maurice T, Privat A. (1997) SA4503, a novel cognitive enhancer with sigma1 receptor agonist properties, facilitates NMDA receptor-dependent learning in mice. *European Journal of Pharmacology*. 328(1):9-18.

Mishra LC, Singh BB, Dagenais S. (2000) Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. *Alternative Medicine Review*. 5(4):334-46.

Mix JA Crews WD Jr (2000)An examination of the efficacy of Ginkgo biloba extract EGb761 on the neuropsychologic functioning of cognitively intact older adults. *Journal of Alternative & Complementary Medicine*. 6(3):219-29

Miyazawa M, Watanabe H, Kameoka H. (1997) Inhibition of acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton. *J. Agric. Food Chem*. 45:677-679.

Miyazawa M, Watanabe H, Umemoto K, Kameoka H. (1998) Inhibition of acetylcholinesterase activity by essential oils of mentha species. *J. Agric. Food Chem*. 46:3431-3434.

Moreau JP, Eck CR, McCabe J, Skinner S. (1986) Absorption, distribution and elimination of a labelled extract of Ginkgo biloba leaves in the rat. *Presse Medicale*. 25;15(31):1458-61

Morris AC, Jacobs I, McLellan TM, Klugerman A, Wang LC, Zamecnik J. (1996) No ergogenic effect of ginseng ingestion. *International Journal of Sport Nutrition* 6(3):263-71

Moss MC, Scholey AB. (1996) Oxygen administration enhances memory formation in healthy young adults. *Psychopharmacology* 124: 255-260.

Moss, M.C., Scholey, A.B., Wesnes, K.A. (1998) Oxygen administration selectively enhances cognitive performance in healthy young adults: a placebo-controlled double-blind crossover study. *Psychopharmacology* 138, 27-33

Moulton PL, Boyko NB, Fitzpatrick JL, Petros TV. (2001) The effect of Ginkgo biloba on memory in healthy male volunteers. *Physiology and Behaviour.* 73: 659-665.

MulkensA, Stephanou E, Kapetenadis I. (1985) Heterosides a genines volatiles dans les feuilles de *Melissa Officinalis* L. (*lamiaceae). Pharmaceutica Acta Helvetiae.* 60:276-278.

Mulnard RI, Cotman CW, Kawas C,Van Dyck CA, Sano H, Doody R, Koss E, Pfeiffer E, Jin S, Gamst A, Grundman M, Thomas R, Mal LJ. (2000) Estrogen replacement therapy for treatment of mild to moderate Alzheimer's disease – A randomised controlled trial. *J. Am. Med. Ass.* 283:1007-1015.

Nathan P. (2000) Can the cognitive enhancing effects of ginkgo biloba be explained by its pharmacology? *Medical Hypotheses.* 55(6):491-3.

Nathan PJ. (2001) Hypericum perforatum (St John's Wort): a non-selective reuptake inhibitor? A review of the recent advances in its pharmacology. *Journal of Psychopharmacology.* 15(1):47-54.

Neri M, Andermarcher E, Pradelli JM, Salvioli G. (1995) Influence of a double blind pharmacological trial on two domains of well being in subjects with age associated memory impairment. 21(3):241-252

Nguyen TT, Matsumoto K, Yamasaki K, Nguyen MD, Nguyen TN, Watanabe H. (1995) Crude saponin extracted from Vietnamese ginseng and its major constituent majonoside-R2 attenuate the psychological stress- and foot-shock stress-induced antinociception in mice. *Pharmacology, Biochemistry & Behavior.* 52(2):427-32.

Nguyen TT, Matsumoto K, Yamasaki K, Nguyen MD, Nguyen TN, Watanabe H. (1996) Effects of majonoside-R2 on pentobarbital sleep and gastric lesion in psychologically stressed mice. *Pharmacology, Biochemistry & Behavior*.53(4):957-63.

Nieder M. (1991) Pharmakokinetic der Ginkgo-flavonole im plasma. *Munch Med Wochenschr.* 133;61-62.

Nishiyama N, Chu PJ, Saito H. (1995b) Beneficial effects of biota, a traditional Chinese herbal medicine on learning impairment induced by basal forebrain-lesion in mice. *Biological & Pharmaceutical Bulletin.* 18(11):1513-7.

Nishiyama N, Chu PJ, Saito H. (1996) An herbal prescription, S-113m, consisting of biota, ginseng and schizandra, improves learning performance in senescence accelerated mouse. *Biological & Pharmaceutical Bulletin.* 19(3):388-93.

Nishiyama N, Wang YL, Saito H. (1995a) Beneficial effects of S-113m, a novel herbal prescription, on learning impairment model in mice. *Biological & Pharmaceutical Bulletin.* 18(11):1498-503.

Nishiyama N, Zhou Y, Saito H. (1994a) Beneficial effects of DX-9386, a traditional Chinese prescription, on memory disorder produced by lesioning the amygdala in mice. *Biological & Pharmaceutical Bulletin.* 17(12):1679-81.

Nishiyama N, Zhou Y, Takashina K, Saito H (1994b) Effects of DX-9386, a traditional Chinese preparation, on passive and active avoidance performances in mice. *Biological & Pharmaceutical Bulletin.* 17(11):1472-6.

Nishiyama N, Zhou Y, Saito H. (1994c) Ameliorative effects of chronic treatment using DX-9386, a traditional Chinese prescription, on learning performance and lipid peroxide content in senescence accelerated mouse. *Biological & Pharmaceutical Bulletin.* 17(11):1481-4.

Nissen MJ, Knopman DS, Schacter DL. (1987) Neurochemical dissociation of memory systems. *Neurology.* 37: 789-794.

Nitta H, Matsumoto K, Shimizu M, Ni XH, Watanabe H. (1995) Panax ginseng extract improves the scopolamine-induced disruption of 8-arm radial maze performance in rats. *Biological & Pharmaceutical Bulletin.*18(10):1439-42

Nordberg A. (1992) Neuroreceptor changes in Alzheimer's disease. Cerebrovasc Brain Metab Rev. 4: 303-328.

Oberpichler H, Beck T, Abdel-Rahman MM, Bielenberg GW, Krieglstein J. (1988) Effects of Ginkgo biloba constituents related to protection against brain damage caused by hypoxia. *Pharmacological Research Communications.* 20(5):349-68

Odani T, Tanizawa H, Takino Y. (1983) Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. IV. Decomposition of ginsenoside-Rg1 and -Rb1 in the digestive tract of rats. *Chemical & Pharmaceutical Bulletin.* 31(10):3691-7

Odani T, Tanizawa H, Takino Y. (1983) Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. III. The absorption, distribution and excretion of ginsenoside Rb1 in the rat. *Chemical & Pharmaceutical Bulletin.* 31(3):1059-66.

Ohnishi Y, Takagi S, Miura T (1996) Effect of ginseng radix on GLUT2 protein content in mouse liver in normal and epinephrine-induced hyperglycemic mice. *Biol Pharm Bull* 19:1238-1240.

Oken BS, Storzbach DM, Kaye JA. (1998) The efficacy of Ginkgo biloba on cognitive function in Alzheimer disease. *Archives of Neurology.* 55(11):1409-15

O'Neill, W.M., Hanks, G.W., White, L., Simpson, P. and Wesnes, K. (1995) The cognitive and psychomotor effects of opioid analgesics I. A randomised controlled trial of single doses of dextropropoxyphrene, lorazepam and placebo in healthy subjects. *European Journal of Clinical Pharmacology.* 48, 447-453

Ong YC, Yong EL (2000) *Panax* (Ginseng)- Panacea or placebo? Molecular and cellular basis of its pharmacological activity. *Ann Acad Med Singapore.* 29: 42-46.

Oshima Y, Sato K, Hikino H (1987) Isolation and hypoglycemic activity of quinquefolans A, B, and C, glycans of Panax quinquefolium roots. *J Nat Prod.* 50:188-190.

Oswald WD, Hoerr R, Oswald B; Steger W, Sappa J. (1997) Increase of fluid cognitive factors with Ginkgo biloba special extract EGb 761(R) in elderly patients with mild to moderate organic brain syndrome. *Zeitschrift-fuer-Gerontopsychologie-and-Psychiatrie..* 10(3): 133-146

Ohta H. Matsumoto K. Shimizu M. Watanabe H. (1994) Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task. *Pharmacology, Biochemistry & Behavior.* 49(1):213-7.

Ohta H. Matsumoto K. Watanabe H. Shimizu M. (1993) Involvement of beta-adrenergic systems in the antagonizing effect of paeoniflorin on the scopolamine-induced deficit in radial maze performance in rats. *Japanese Journal of Pharmacology.* 62(4):345-9.

Park CH, Kim SH, Choi W, Lee YJ, Kang S, Suh YH. (1996) Novel anticholinesterase and anti-amnesic activities of Evodia rutaecarpa. *Planta Medica.* 62:405-409.

Park KM, Kim YS, Jeong TC, Joe CO, Shin HJ, Lee YH, Nam KY, Park JD. (2001) Nitric oxide is involved in the immunomodulating activities of acidic polysaccharide from Panax ginseng. *Planta Medica.* 67(2):122-6.b

Park YC, Lee CH, Kang HS, Kim KW, Chung HT, Kim HD. (1996) Ginsenoside-Rh1 and Rh2 inhibit the induction of nitric oxide synthesis in murine peritoneal macrophages. *Biochemistry & Molecular Biology International.* 40(4):751-7

Parsons MW, Gold PE. (1992) Glucose enhancement of memory in elderly humans: an inverted-U dose response curve. *Neurobiology of Ageing.* 13:401-404.

Parys W. (1998) Development of Reminyl (Galantamine) a novel acetylcholinesterase inhibitor for the treatment of Alzheimer's disease. *Alzheimer's Report.* 1: S19-S20.

Peng CF, Li YJ, Li YJ, Deng HW. (1995) Effects of ginsenosides on vasodilator nerve actions in the rat perfused mesentery are mediated by nitric oxide. *Journal of Pharmacy & Pharmacology.* 47(7):614-7.

Perry EK. (1986) The cholinergic hypothesis ten years on. *British Medical Bulletin.* 42: 63-69.

Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NSL (1999) Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *Journal of Pharmacy and Pharmacology* 51:527-534.

Perry N, Court G, Bidet N, Court J, Perry E (1996) European Herbs with cholinergic activities: potential in dementia therapy. *International Journal of Geriatric Psychiatry.* 11: 1063-1069.

Perry NSL, Houghton PJ, Jenner P, Keith A, Perry EK. (2002) Salvia lavandulaefolia essential oil inhibits cholinesterase in vivo. *Phytomedicine.* 9: 48-51.

Perry NSL, Houghton PJ, Sampson J, Theobald AE, Hart S, Lis-Balchin M, Hoult JRS, Evans P, Jenner P, Milligan S, Perry EK. (2001) In-vitro activities of S, lavandulaefolia (Spanish Sage) relevant to treatment of Alzheimer's disease. *Journal of Pharmacy and Pharmacology.* 53:1347-1356.

Perry NSL, Houghton P, Theobald A, Jenner P, Perry EK. (2000) In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia Lavandulaefolia essential oil and constituent terpenes. *Journal of Pharmacy and Pharmacology.* 52:895-902.

Perry N, Howes M, Houghton P, Perry E. (2000) Why Sage may be a wise remedy: Effects of Salvia on the nervous system. In Kintzios SE. (Editor) *Sage: The genus Salvia.* Harwood Academic Publishers. Amsterdam.

Peters H, Kieser M, Holscher U. (1998) Demonstration of the efficacy of ginkgo biloba special extract EGb 761 on intermittent claudication--a placebo-controlled, double-blind multicenter trial. *VASA.* 27(2):106-10

Peterson RC (1977) Scopolamine induced learning failures in man. *Psychopharmacology.* 52: 283-289.

Petkov V. (1978) Effect of ginseng on the brain biogenic monoamines and 3',5'-AMP system. Experiments on rats. *Arzneimittel-Forschung.* 28(3):388-93

Petkov VD, Getova D, Mosharrof AH. (1987) A study of nootropic drugs for anti-anxiety action. *Acta Physiologica et Pharmacologica Bulgarica.* 13(4):25-30

Petkov VD, Mosharrof AH. (1987) Effects of standardized ginseng extract on learning, memory and physical capabilities. *American Journal of Chinese Medicine.* 15(1-2):19-29

Petkov VD, Kehayov R, Belcheva Konstantinova E, Petkov VV, Getova D, Markovska V. (1993) Memory effects of standardized extracts of Panax ginseng (G115),Ginkgo biloba (GK 501) and their combination Gincosan (PHL-00701). *Planta Medica.* 59(2):106-14

Petkov VD, Cao Y, Todorov I, Lazarova M, Getova D, Stancheva S, Alova L.(1992) Behavioral effects of stem-leaves extract from panax ginseng C.A. Meyer.. *Acta Physiologica et Pharmacologica Bulgarica.* 18(2):41-8

Phillipson JD, Anderson LA. (1984) Ginseng- quality safety and efficacy? *Pharmaceut Journal.* 232:161-5.

Pidoux B, Bastien CI, Niddam S. (1983) Clinical and Quantitative EEG double-blind study of *Ginkgo Biloba* extract.. *Journal of Cerebral Blood Flow and Metabolism.* 3: s556-s557

Pidoux B. (1986) Effects of *Ginkgo biloba* extract on functional brain activity.. An assessment of clinical and experimental studies. *Presse Medicale.* 25;15(31):1588-91

Pieralisi G, Ripari P, Vecchiet L. (1991) Effects of a standardized ginseng extract combined with dimethylaminoethanol bitartrate, vitamins, minerals, and trace elements on physical performance during exercise. *Clinical Therapeutics.* 13(3):373-82

Pietta PG, Gardana C, Mauri PL. (1997) Identification of Gingko biloba flavonol metabolites after oral administration to humans. *J Chromatogr B Biomed Sci Appl.* 693(1):249-55.

Pittler MH, Ernst E (2000) Ginkgo biloba extracrt for the treatment of intermittent Claudication: A meta-analysis of randomised trials. *The American Journal of Medicine.* 108: 276-281.

Polster MR. (1993) Drug-induced amnesia: implications for cognitive neuropsychological investigations of memory. *Psychological Bulletin.* 114:477-493.

Porsolt RD, Martin P, Lenegre A, Fromage S, Drieu K. (1990) Effects of an extract of Ginkgo Biloba (EGB 761) on "learned helplessness" and other models of stress in rodents. *Pharmacology, Biochemistry & Behavior.* 36(4):963-71

Prast H, Philippu A. (2001) Nitric oxide as modulator of neuronal function. *Progress in Neurobiology.* 64(1):51-68.

Prehn JH. Krieglstein J. (1993) Platelet-activating factor antagonists reduce excitotoxic damage in cultured neurons from embryonic chick telencephalon and protect the rat hippocampus and neocortex from ischemic injury in vivo. *Journal of Neuroscience Research.* 34(2):179-88.

Price, S. (1998). *Aromatherapy Workbook.* Harper Collins, London.

Rai GS, Shovlin C, Wesnes KA. (1991) A double-blind, placebo controlled study of Ginkgo biloba extract ('tanakan') in elderly outpatients with mild to moderate memory impairment. *Current Medical Research & Opinion.* 12(6):350-5

Ramassamy C, Averill D, Beffert U, Bastianetto S, Theroux L, Lussier-Cacan S, Cohn JS, Christen Y, Davignon J, Quirion R, Poirier J (1999) Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease is related to the Apolipoprotein E genotype. *Free Radical Biology and Medicine.* 27: 544-553.

Ramassamy C, Christen Y, Clostre F, Costentin J. (1992a) The Ginkgo biloba extract, EGb761, increases synaptosomal uptake of 5-hydroxytryptamine: in-vitro and ex-vivo studies. *Journal of Pharmacy & Pharmacology.* 44(11):943-5

Ramassamy C, Girbe F, Christen Y,Costentin J. (1993) Ginkgo biloba extract EGb 761 or trolox C prevent the ascorbic acid/Fe2+ induced decrease in synaptosomal membrane fluidity. *Free Radical Research Communications.* 19(5):341-50

Ramassamy C, Naudin B, Christen Y, Clostre F, Costentin J. (1992b) Prevention by Ginkgo biloba extract (EGb 761) and trolox C of the decrease in synaptosomal dopamine or serotonin uptake following incubation. *Biochemical Pharmacology*. 15;44(12):2395-401

Rapin JR, Le Poncin, Lafitte M. (1986) Cerebral glucose consumption. The effect of Ginkgo biloba extract. *Presse Medicale*. 15(31):1494-7

Rapin JR, Lamproglou I, Drieu K, DeFeudis FV. (1994) Demonstration of the "anti-stress" activity of an extract of Ginkgo biloba (EGb 761) using a discrimination learning task. *General Pharmacology*. 25(5):1009-16

Rawls, R. (1996) Europe's strong herbal brew: Chemical and biological research, mostly from Europe, supports the growing respectability of herbal medicines in US. *Chem Eng. N.*, September 23: 53-60

Reddy DS, Kulkarni SK. (1998) Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging- and dizocilpine-induced learning impairment. *Brain Research*. 799(2):215-29.

Rigney U, Kimber S, Hindmarch I. (1999) The effects of acute doses of standardised Ginkgo biloba extract on memory and psychomotor performance in volunteers. Phytotherapy Research. 13(5):408-15.

Rivera D, Obon C, Cano R. (1994) The botany, history and traditional uses of Three-lobed Sage (*Salvia Fruticosa* Miller)(Labiatae). *Economic Botany*. 48(2):190-195.

Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT (1998) A 24 week, double blind, placebo controlled trial of donepezil in patients with Alzheimer's disease. *Neurology*. 50: 136-145.

Roncin JP, Schwartz F, D'Arbigny P. (1996) Egb761 in control of acute mountain sickness and vascular reactivity to cold exposure. Aviation, *Space and Environmental medicine*. 67(5)445-452.

Roteliste (2001) CMDI/Boehringer Ingelheim.

Roy D, Perrault M, Marette A. (1998) Insulin stimulation of glucose uptake in skeletal muscle and adipose tissue in vivo is NO dependent. *Am J Physiol.* 274:E692-E699

Russo E. (2001) *Handbook of psychotropic herbs.A scientific analysis of herbal remedies for psychiatric conditions*. Howarth Herbal Press. New York.

Rusted, J.M. and Warburton, D.M. (1989) Cognitive models and cholinergic drugs. *Neuropsychobiology.* **21,** 31-36

Rusted JM (1988) Dissociative effects of of scopolamine on working memory in healthy young volunteers. *Psychopharmacology*, 96 : 487-492.

Rusted JM, Eaton-Williams P, Warburton DM. (1991) A comparison of the effects of scopolamine and diazepam on working memory. *Psychopharmacology*. 105 : 442-445.

Rusted JM, Warburton DM (1988) Effects of scopolamine on working memory in healthy young volunteers. *Psychopharmacology*. 96 : 145-152.

Rusted, J.M. Warburton, D.M. (1991) Molecules for modelling cognitive impairment. In. Hindmarch I, Hippius H, Wilcox G (Eds) Dementia, molecules, methods and measurement. Academic Press. London Harwood.

Ryman D. (1991) *Aromatherapy.* Judy Piakus Publishers, London. Pp.190-194.

Saito H, Tsuchiya M, Naka S, Takagi K. (1977) Effects of Panax Ginseng root on conditioned avoidance response in rats. *Japanese Journal of Pharmacology*. 27(4):509-16

Salemme E, Diano S, Maharajan P, Maharajan V. (1996) Nitric oxide, a neuronal messenger. Its role in the hippocampus neuronal plasticity. *Rivista di Biologia.* 89(1):87-107.

Salim KN, McEwen BS, Chao HM. (1997) Ginsenoside Rb1 regulates ChAT, NGF and trkA mRNA expression in the rat brain. Brain Research. *Molecular Brain Research.* 47(1-2):177-82.

Satyan KS, Jaiswal AK, Ghosal S,Bhattacharya SK. (1998) Anxiolytic activity of ginkgolic acid conjugates from Indian Ginkgo biloba. *Psychopharmacology*. 136(2):148-52

Savel J. (1971) *Pharmacological investigation of the standardised ginseng extract G115.* Delrray Beach. Ginsana USA corporation,.

Scaglione F, Cattaneo G, Alessandria M, Cogo R. (1996) Efficacy and safety of the standardised ginseng extract G115 for potentiating vaccination against the infuenza syndrome and protection against the common cold. *Drugs Exp Clin Res*. 22:65-72.

Schaffler K, Reeh PW. (1985) Double blind study of the hypoxia protective effect of a standardized Ginkgo biloba preparation after repeated administrationin healthy subjects. *Arzneimittel-Forschung*. 35(8):1283-6

Schliebs R, Liebmann A, Bhattacharya SK, Kumar A, Ghosal S, Bigl V. (1997) Systemic administration of defined extracts from Withania somnifera (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and GABAergic markers in rat brain. *Neurochemistry International*. 30(2):181-90.

Scholey. AB (2001) Fuel for thought. *The Psychologist.* 14(4):196-201.

Scholey AB. (2002) Attention. In Perry EK, Ashton H, Young A. (Eds) *Neurochemistry of consciousness*. 43-63. John Benjamin. Amsterdam.

Scholey AB, Harper S, Kennedy DO. 2001. Cognitive demand and blood glucose. *Physiol Behav*. 73(4):585-92

Scholey, A.B., Moss, M.C., Neave, N. and Wesnes, K.A. (1999) Cognitive performance, hyperoxia and heart rate following oxygen administration in healthy young adults. *Physiology and Behavior* 67, 783-789

Schulz H, Jobert M, Breuel HP (1991) Wirkung von Spezialextrakt LI1370 auf das EEG älterer Patienten im Schlafentzugs-Modell. *Munch. Med. Wschr.* 133: S26-S29.

Schweizer J, Hautmann C (1999) Comparison of two dosages of Ginkgo biloba extract Egb 761 in patients with peripheral arterial occlusive disease Fontaine's stage II. Arzneim.-Forsch 49: 900-904.

Scimone, A. (1997) Phytochemicals taking their place. *Chem. Mark. Rep.*, 251(24) (June 16), sr21-sr22

Scimone, A. & Scimone, A. (1998) US sees the green in herbal supplements. *Chem. Mark. Rep.*, (July 13), fr3-fr4

Semlitsch HV, Anderer P, Saletu B, Binder GA, Decker KA. (1995) Cognitive psychophysiology in nootropic drug research: effects ofGinkgo biloba on event-related potentials (P300) in age-associated memory impairment. *Pharmacopsychiatry*. 28(4):134-42

Shepherd JE. (2001) Effects of estrogen on cognition, mood, and degenerative brain diseases. *Journal of the American Pharmaceutical Association*. 41(2):221-8..

Shim I, Won J, Lee J, Song D, Kim SE, Huh S, Kim Y, Suh H. (2000) Modulatory effect of Ginseng total saponin on dopamine release and tyrosine hydroxylase gene expression induced by nicotine in the mouse. *Journal of Ethnopharmacology*. 70:161-169.

Shi L, Fan PS, Wu L, Fang JX, Han ZX. (1990) Effects of total saponins of Panax notoginseng on increasing PGI2 in carotid artery and decreasing TXA2 in blood platelets. *Chung-Kuo Yao Li Hsueh Pao - Acta Pharmacologica Sinica*. 11(1):29-32

Siddique MS, Eddeb F, Mantle D, Mendelow AD. (2000) Extracts of Ginkgo biloba and Panax Ginseng protect brain proteins from free radical induced oxidative damage in vitro. *Acta Neurochir*. 76:87-90, 2000

Siegel RK. (1979) Ginseng abuse syndrome. Problems with the panacea. *JAMA*. 241(15):1614-5.

Singh B, Saxena AK, Chandan BK, Gupta DK, Bhutani KK, Anand KK. (2001) Adaptogenic activity of a novel, withanolide-free aqueous fraction from the roots of Withania somnifera Dun. *Phytotherapy Research*. 15(4):311-8.

Singh HK, Dhawan BN. (1982) Effect of Bacopa monniera Linn. (brahmi) extract on avoidance responses in rat. *Journal of Ethnopharmacology*. 5(2):205-14.

Sloley BD, Pang PK, Huang BH, Ba F, Li FL, Benishin CG, Greenshaw AJ, Shan JJ. (1999) American ginseng extract reduces scopolamine induced amnesia in a spatial learning task. Journal of Psychiatry and Neuroscience. 24(5):442-52.

Smith PF, Maclennan K, Darlington CL.(1996)The neuroprotective properties of the Ginkgo biloba leaf: a review of the possible relationship to platelet-activating factor (PAF). Journal of Ethnopharmacology. 50(3):131-9

Smith RG, Caswell D, Carriere A **et al** (1996) Variation in the ginsenoside content of American ginseng, Panax quinquefolius L roots. *Canadian Journal of Botany*. 74:1616-1620.

Smriga M, Saito H, Nishiyama N. (1995) Hoelen (Poria Cocos Wolf) and ginseng (Panax Ginseng C. A. Meyer), the ingredients of a Chinese prescription DX-**9386**, individually promote hippocampal long-term potentiation in vivo. *Biological & Pharmaceutical Bulletin.* 18(4):518-22.

Soldati F, Sticher O. (1980) HPLC separation and quantitative determination of ginsenosides from Panax ginseng, Panax quinquefolium and from ginseng drug preparations. 2nd communication. *Planta Medica*. 39(4):348-57.

Sonnenborn U, Proppert Y (1990) Ginseng (Panax Ginseng C A Meyer). *Z Phytother*. 11: 35-49

Sorensen H, Sonne J. (1996) A double masked study of the effects of ginseng on cognitive functios. *Current Therapeutic Research*. 57(12):959-968

Sotaniemi EA, Haapakoski E, Rautio A. (1995) Ginseng therapy in non-insulin-dependent diabetic patients. *Diabetes Care*. 18(10):1373-5

Soulimani R, Younos C, Fleurentin J, Mortier F, Misslin R, Derrieu G. (1993) Recherche de L'activite biologique de *Melissa officinalis* L. sur le systeme nerveux central de la souris in vivo et le duodenum de rat in vitro; *Plantes Med. Phytother*. 26,2: 77-85.

Soulimani R, Fleurentin J, Mortier F, Misslin R, Derrieu G, Pelt J.M. (1991) Neurotropic action of the hydroalcoholic extract of Melissa officinalis in the mouse; *Planta Medica.* **57**;105-109

Spinas GA, Laffranchi R, Francoys I, David I, Richter C, Reinecke M. (1998) The early phase of glucose-stimulated insulin secretion requires nitric oxide. *Diabetologia.* 41:292-299

Spinnewyn B, Blavet N, Clostre F. (1986) Effects of Ginkgo biloba extract on a cerebral ischemia model in gerbils. *Presse Medicale.* 15(31):1511-5

Springen K. Cowley G. (1997) Brain boosters. Sale of ginkgo biloba, natural memory booster, climb to over $100 mil in 1996. *Newsweek*, (November 3), 58

Steriade M, McCormick DA, Sejnowski TJ. (1993) Thalamocortical oscillations in the sleeping and aroused brain. *Science.* 262 (5134): 679-685

Stoll S, Scheuer K, Pohl O, Muller WE. (1996) Ginkgo biloba extract (EGb 761) independently improves changes in passive avoidance learning and brain membrane fluidity in the aging mouse. *Pharmacopsychiatry.* 29(4):144-9

Stone WS, Walser B, Gold SD, Gold PE. (1991) Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: reversal with glucose and with cholinergic and adrenergic agonists. *Behavioural Neuroscience.* 105(2): 264-271.

Sung J, Han KH, Zo JH, Park HJ, Kim CH, Oh BH.(2000) Effects of red ginseng upon vascular endothelial function in patients with essential hypertension. *American Journal of Chinese Medicine.* 28(2):205-16.

Sunram-Lea SI, Foster JK. (2002) Glucose and memory: a Neurocognitive Review. *Psychopharmacology. In Press.*

Sunram-Lea SI, Foster JK, Durlach P, Perez C.(2002)Investigation into the significance of task difficulty and divided allocation of resources on the glucose memory facilitation effect. *Psychopharmacology* . 160: 387-397

Suzuki Y, Ito Y, Konno C, Furuya T. (1991) Effects of tissue cultured ginseng on gastric secretion and pepsin activity. *Yakugaku Zasshi.* 111:770-774

Tabata K, Matsumoto K, Watanabe H. (2000) Paeoniflorin, a major constituent of peony root, reverses muscarinic M1-receptor antagonist-induced suppression of long-term potentiation in the rat hippocampal slice. *Japanese Journal of Pharmacology.* 83(1):25-30.

Tachikawa E, Kudo K, Harada K, Kashimoto T, Miyate Y, Kakizaki A, Takahashi E. (1999) Effects of ginseng saponins on reponses induced by various receptor stimuli. *European Journal of Pharmacology.* 369: 23-32

Tadano T, Nakagawasai O, Tan-no K, Morikawa Y, Takahashi N, Kisara K. (1998) Effects of ginkgo biloba extract on impairment oflearning induced by cerebral ischemia in mice. *American Journal of Chinese Medicine.* 26(2):127-32

Takagi K, Saito H, Tsuchiya M. (1972a) Pharmacological studies of Panax Ginseng root: pharmacological properties of a crude saponin fraction. *Japanese Journal of Pharmacology.* 22(3):339-46

Takagi K, Saito H, Nabata H. (1972b) Pharmacological studies of Panax ginseng root:estimation of pharmacological actions of Panax ginseng root. *Japanese Journal of Pharmacology.* 22(2):245-9

Tagashira M, Ohtake Y. (1998) A new antioxidative 1,3-benzodioxole from Melissa Officinalis. *Planta Medica.* 64(6):555-558.

Tamada J A, Garg S, Jovanovic L, Pitzer K R, Fermi S, Potts R O. (1999) Non-invasive glucose monitoring: comprehensive clinical results. Cygnus Research Team ***JAMA.*** 282:1839-1844.

Tamaoki J, Nakata J, Kawatani K, Tagaya E, Nagai A. (2000) Ginsenoside-induced relaxation of human bronchial smooth muscle via release of nitric oxide. *British Journal of Pharmacology.* 130(8):1859-64.

Taylor JE. (1986) Neuromediator binding to receptors in the rat brain. The effect of chronic administration of Ginkgo biloba extract. Presse Medicale. 15(31):1491-3

Taylor LA, Rachman SJ. (1987) The effects of blood sugar levels changes on cognitive function, affective state and somatic symptoms. *J Behav Med.* 20: 544-549.

Teng CM, Kuo SC, Ko FN, Lee JC, Lee LG, Chen SC, Huang TF. (1989) Antiplatelet actions of panaxynol and ginsenosides isolated from ginseng. *Biochimica et Biophysica Acta.* 990(3):315-20

Thommessen B, Laake K. (1996) No identifiable effect of ginseng (Gericomplex) as an adjuvant in the treatment of geriatric patients. *Aging.* 8(6):417-20

Tierney MJ, Tamada JA, Potts RO, Jovanovic L, Garg S. (2001) Clinical evaluation of the GlucoWatch biographer: a continual, non-invasive glucose monitor for patients with diabetes. *Biosensors and Bioelectronics.* 16; 621-629.

Tittel G, Wagner H, Bos R, (1982) Über die chemische Zusammensetzung von Melisseolen; *Planta Med.* 46; 91-98.

Toda N, Ayajiki K, Fujioka H, Okamura T. (2001) Ginsenoside potentiates NO-mediated neurogenic vasodilatation of monkey cerebral arteries. *Journal of Ethnopharmacology.* 76(1):109-13.

Tode T, Kikuchi Y, Hirata J, Kita T, Nakata H, Nagata I.. (1999) Effects of Korean red ginseng on psychological functions in patients with severe climacteric syndromes. *International Journal of Gynecology and Obstetrics.* 67:169-174.

Topp S, Knoefel WT, Schutte S, Brilloff S, Rogiers X, Gubdlach M. (2001) Ginkgo biloba (Egb761) improves microcirculation after warm water ischemia of the rat liver. *Transplantation proceedings.* 33: 979-981.

Tripathi YB, Chaurasia S, Tripathi E, Upadhyay A, Dubey GP. (1996) Bacopa monniera Linn. as an antioxidant: mechanism of action. *Indian Journal of Experimental Biology.* 34(6):523-6.

Ussher JM, Dewberry C, Malson H, Noakes J. (1995) The relationship between health related quality of life and dietary supplementation in british middle managers: A double blind placebo controlled study. *Psychology and Health.* 10(2):97-111.

Valnet J. (1990) *Aromatherapie.* Maloine. S.A. Publishers. Paris.

Van Dongen MCJM, van Rossum E, Kessels AGH, Sielhorst HJG, Knipschild PG. (2000) The efficacy of Ginkgo for elderly people with dementia and age associated memoy impairment: New results of a randomised clinical trial. *Journal of the American Geriatrics Society.* 28: 1183-1194.

Varga E, Bodi A, Ferdinandy P, Droy-Lefaix MT, Blasig IE, Tosaki A. (1999) The protective effect of EGb 761 in isolated ischemic/reperfused rat hearts: a link between cardiac function and nitric oxide production. *Journal of Cardiovascular Pharmacology.* 34(5):711-7.

Vaughan MA, Doolittle RL, Gennett T. (1999) Physiological effects of ginseng may be due to methylxanthines. *Med Sci Sports Exercise.* 31:S121.

Vinekar AS, Andrade C, Sriprada VT, Gearge J, Joseph T, Chandra JS. (1998) Attenuation of ECS-induced retrograde amnesia by using an herbal formulation. *Journal of ECT.* 14(2):83-8.

Vogler BK, Pittler MH, Ernst E. The efficacy of Ginseng. A systematic review of randomised clinical trials. *European Journal of Clinical Pharmacology..*1999. 55(8): 567-575.

Vuksan V, Sievenpiper JL, Koo VYY, Francis T, Beljan-Zdravkovic U, Xu Z, Vidgen E. (2000a) American Ginseng (Panax quinquefolius L) Reduces Postprandial Glycemia in Nondiabetic Subjects and Subjects With Type 2 Diabetes Mellitus. *Archives of Internal Medicine.* 160(7):1009-1013

Vuksan V, Stavro MP, Sievenpiper JL, Beljan-Zdravkovic U, Leiter LA, Josse RG, Xu Z. (2000b) Similar postprandial glycemic reductions with escalation of dose and administration time of American ginseng in type 2 diabetes. *Diabetes Care.* 23(9):1221-6.

Vuksan V. Sievenpiper JL. Wong J. Xu Z. Beljan-Zdravkovic U. Arnason JT. Assinewe V. Stavro MP. Jenkins AL. Leiter LA. Francis T. (2001) American ginseng (Panax quinquefolius

L.) attenuates postprandial glycemia in a time-dependent but not dose-dependent manner in healthy individuals. *American Journal of Clinical Nutrition*. 73(4):753-8.

Wagner H, Sprinkmeyer L, (1973) Über die pharmakologische Wirkung von Melissengeist; *Dtsch. Apoth. Ztg.* 113; 1159-66.

Wake G, Court J, Pickering A, Lewis R, Wilkins R Perry E (2000) CNS acetylcholine receptor activity in European medicinal plants traditionally used to improve failing memory. *Journal of Ethnopharmacology*. 69: 105-114.

Wang A, Cao Y, Wang Y, Zhao R, Liu C. (1995) Effects of Chinese ginseng root and stem-leaf saponins on learning, memory and biogenic monoamines of brain in rats. *Chung-Kuo Chung Yao Tsa Chih - China Journal of Chinese Materia Medica*. 20(8):493-5.

Wang LC, Lee TF. (1998) Effect of ginseng saponins on exercise performance in non-trained rats. *Planta Med.* 64(2):130-3

Ward T, Wesnes K. (1999) Validity and utility of the CDR computerised cognitive assessment system: a review following 15 years of use. *J Psychopharmacology*. 2:A25

Warot D, Lacomblez L, Danjou P, Weiller E, Payan C, Puech AJ. (1991) Comparative effects of ginkgo biloba extracts on psychomotor performances and memory in healthy subjects. *Therapie*. 46(1):33-6

Watanabe H, Ni JW, Ohia H, Ni X, Matsumoto K. (1991) A Kampo prescription, shimotsu-to, improves scopolamine induced spatial cognitive deficits in rats. *Japanese Journal of Psychopharmacology*. 11: 215-222.

Watanabe (1993) Psychotropic effects of Sino-Japanese traditional medicines. *Japanese Journal of Psychopharmacology*. 13(2):51-7.

Welt K, Fitzl G, Schaffranietz L. (1996) Myocardium-protective effects of Ginkgo biloba extract (EGb 761) in old rats against acute isobaric hypoxia. An electron microscopic morphometric study. II. Protection of microvascular endothelium. *Experimental & Toxicologic Pathology*. 48(1):81-6

Wen TC, Yoshimura H, Matsuda S, Sakanaka M. (1996) Ginseng root prevents learning disability and neuronal loss in gerbils with 5-minute forebrain ischemia.. *Acta Neuropathologica.* 91(1):15-22

Wenk GL. (1989) A hypothesis of the role of glucose in the mechanism of cognition enhancers. *Psychopharmacology* 99:431-438.

Wesnes K, Anand R, Simpson P, Christmas L. (1990) The use of the scopolamine model to study the potential nootropic effects of aniracetam and piracetam in healthy volunteers. *Journal of Psychopharmacology.* 4 (4): 219-232.

Wesnes KA, Faleni RA, Hefting NR, Hoogsteen G, Houben JJG, Jenkins E, Jonkman JHG, Leonard J, Petrini O, van Lier JJ. (1997) The cognitive, subjective, and physical effects of a *Ginkgo biloba / Panax ginseng* combination in healthy volunteers with neurasthenic complaints. *Psychopharm Bull.* 33:677-683

Wesnes, K.A.; McKeith IG, Ferrara R, Emre M, Del Ser T, Spano PF, Cicin-Sain A, Anand R, Spiegel R. (2002) Effects of Rivastignine on cognitive function in Dementia with Lewy Bodies: a randomized, placebo controlled, international study using the Cognitive Drug Research computerized assessment system. *Dementia and Geriatric Cognitive Disorders.* In Press.

Wesnes K, Simmons D, Rook M, Simpson P. (1987a) A double blind placebo controlled trial of Tanakan in the treatment of idiopathic cognitive impairment in the elderly. *Human Psychopharmacology.* 2: 159-169.

Wesnes, K., Simpson, P.M. and Christmas, L. (1987b) *in The assessment of human information processing abilities in psychopharmacology: measures and methods, Vol. 1.* Wiley, Chichester

Wesnes, K., Simpson, P.M. and Kidd, A.G. (1988) An investigation of the range of cognitive impairments induced by scopolamine 0.6 mg. *Human Psychopharmacology* **3,** 27-43

Wesnes K, Warburton DM, Matz B. (1983) Effects of nicotine on stimulus sensitivity and response bias in a visual vigilance task. *Neuropsychobiology,* 9 : 41-44.

WesnesK, Warburton DM. (1984) Effects of scopolamine and nicotine on human rapid information processing performance. *Psychopharmacology,* 82 : 147-150.

Wesnes KA, Ward T, McGinty A, Petrini O. (2000) The memory enhancing effects of a Ginkgo-biloba/Panax ginseng combination in healthy middle aged volunteers. *Psychopharmacology.* 152: 353-361.

White HL, Scates PW, Cooper BR. (1996) Extracts of Ginkgo biloba leaves inhibit monoamine oxidase. *Life Sciences.* 58(16):1315-21

Wiklund I, Karlberg J, Lund B. (1994) A double blind comparison of the effects on quality of life of a combination of vital substances including standardised ginseng G115 and placebo. *Current Therapeutic Research.* 55(1): 32-42.

Wiklund IK, Mattsson LA, Lindgren R, Limoni C. (1999) Effects of a standardised ginseng extract on quality of life and physiological parameters in symptomatic postmenopausal women: a double blind, placebo-controlled trial. Swedish Alternative Medicine Group. *Int J Clin Pharmacol Res.* 19(3):89-99.

Wilcock GK. Lilienfeld S. Gaens E. (2000) Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: multicentre randomised controlled trial. Galantamine International-1 Study Group. *BMJ.* 321(7274):1445-1449.

Wilke, M. (1997) *Bob Carraher, Ginsana.* http;//www.adage.com/news_and_features/s... rts/mktg.

Winter E.. (1991) Effects of an extract of Ginkgo biloba on learning and memory in mice. *Pharmacology, Biochemistry & Behavio*r. 38(1):109-14

Winter JC. (1998) The effects of an extract of Ginkgo biloba, EGb 761, on cognitive behavior and longevity in the rat. *Physiology & Behavior.* 63(3):425-33

Witte S, Anadere I, Walitza E. (1992) Improvement of hemorheology with ginkgo biloba extract. Decreasing a cardiovascular risk factor. *Fortschritte der Medizin.* 110(13):247-50

Wong AHC, Smith M, Boon HS. (1998) Herbal remedies in psychiatric practice. *Archive of General Psychiatry*. 55:1033-1044.

Wood J, Garthwaite J. (1994) Models of the diffusional spread of nitric oxide: implications for neural nitric oxide signalling and its pharmacological properties. *Neuropharmacology*. 33: 1235-1244.

Wood WB, Roh BL, White RP. (1964) Cardiovascular actions of panax ginseng in dogs. *Jpn J Pharmacol*. 14:284-294

Wu CR, Hsieh MT, Huang SC, Peng WH, Chang YS, Chen CF. (1996a) Effects of Gastrodia elata and its active constituents on scopolamine-induced amnesia in rats. *Planta Medica*. 62(4):317-21.

Wu CR, Hsieh MT, Liao J. (1996b) p-Hydroxybenzyl alcohol attenuates learning deficits in the inhibitory avoidance task: involvement of serotonergic and dopaminergic systems. *Chinese Journal of Physiology*. 39(4):265-73.

Xin W, Wei T, Chen C, Ni Y, Zhao B, Hou J. (2000) Mechanisms of apoptosis in rat cerebellar granule cells induced by hydroxyl radicals and the effects of Egb 761 and its constituents. *Toxicology*. 148:103-110.

Yamaguchi Y, Haruta K, Kobayashi H, (1995) Effects of ginsenosides on impaired performance induced in the rat by scopolamine in a radial arm maze. *Psychoneuroendocrinology*. 20:645-653.

Yamaguchi Y, Higashi M, Kobayashi H, (1996) Effects of ginsenosides on impaired performance caused by scopolamine in the rat. *European Journal of Pharmacology*. 312: 149-151..

Yao Z, Drieu K, Papadopoulos V. (2001) The Ginkgo biloba extract Egb 761 rescues the PC12 neuronal cells from β-Amyloid induced cell death by inhibiting the formation of β-Amyloid derived diffusible neurotoxic ligands. *Brain Research*. 889:181-190.

Yao Z, Drieu K, Szweda LI, Papadopoulos V (1999) Free radicals and Lipid peroxidation do not mediate the β-Amyloid induced neuronal cell death. *Brain Research.* 847:: 203-210.

Yobimoto K, Matsumoto K, Huong NTT, Kasai R, Yamasaki K, Watanabe H. (2000) Suppressive effects of Vietnamese ginseng saponin and its major component majonoside R2 on psychological stress-induced enhancement of lipid peroxidation in the mouse brain. *Pharmacology Biochemistry and Behaviour.* 66: 661-665

Yuan CS, Wu JA, Lowell T, Gu M. (1998) Gut and brain effects of American ginseng root on brainstem neuronal activities in rats. *Am J Chin Med.* 26:47-55

Yun TK, Choi SY (1995) Preventive effects of ginseng intake against various human cancers: a case control study on 1987 pairs. *Cancer Epedemiol Biomarkers Prev.* 4:401-408

Yun TK, Choi SY (1998) Non-organ specific cancer prevention by ginseng: a prospective study in Korea. *International Journal of Epidemiology.* 27:359-364.

Zhan Y, Xu XH, Jiang YP. (1994) Effects of ginsenosides on myocardial ischemia/reperfiusion damage in open-heart surgery patients. *Med J China.* 74:626-628

Zhang YG, Liu TP. (1996) Influences of ginsenosides Rb1 and Rg1 on reversible focal brain ischemia in rats. *Acta Pharmacologica Sinica.* 17(1):44-8.

Zhang D, Yasuda T, Yu Y, Zheng P, Kawabata T, Ma Y, Okada S. (1996) Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. Free Radical Biology & Medicine. 20(1):145-50.

Zhang JT, Qu ZW, Liu Y, Deng HL.(1990) Preliminary study on antiamnestic mechanism of ginsenoside Rg1 and Rb1.*Chinese Medical Journal.* 103(11):932-8.

Zhang Y, Saito H, Nishiyama N. (1994) Improving effects of DX-9386, a traditional Chinese medicinal prescription, on thymectomy-induced impairment of learning behaviors in mice. *Biological & Pharmaceutical Bulletin.* 17(9):1199-205.

Zhang Y, Saito H, Nishiyama N, Abe K. (1994) Effects of DX-9386, a traditional Chinese medicinal prescription, on long-term potentiation in the dentate gyrus in rats. *Biological & Pharmaceutical Bulletin.* 17(10):1337-40.

Zhao R, McDaniel K. (1998) Ginseng improves strategic learning by normal and brain-damaged rats. *Neuroreport.* 9(7):1619-24

Zhong GG, Sun CW, Li YY, Qi H, Zhao CY, Jiang Y, Wang XM, Yang SJ, Li H. (1995) Calcium channel blockade and anti-free-radical actions of panaxadiol saponins Rb1, Rb2, Rb3, Rc, and Rd. *Chung-Kuo Yao Li Hsueh Pao - Acta Pharmacologica Sinica.* 16(3):255-60

Zhong Y, Nishijo H, Uwano T, Tamura R, Kawanishi K, Ono T. (2000) Red ginseng ameliorated place navigation deficits in young rats with hippocampal lesions and aged rats. *Physiology and Behaviour.* 69: 511-525.

Zuin M, Battezzati PM, Camisasca M, Riebenfeld D, Podda M. (1987) Effects of a preparation containing a standardised ginseng extract combined with trace elements and multi-vitamins against hepatotoxin- induced chronic liver disease in the elderly. *Journal of International Medical Research.* 15:276-281.

# Appendices

**Appendix I** Identification of a melissa officinalis (dried leaf) with human CNS nicotinic and muscarinic binding properties

**Appendix II** Bond-Lader visual analogue scale

# APPENDIX I.

## IDENTIFICATION OF A *MELISSA OFFICINALIS* (DRIED LEAF) WITH HUMAN CNS NICOTINIC AND MUSCARINIC RECEPTOR BINDING PROPERTIES

### Introduction

In comparison to a previous investigation of a number of accessions of *M. officinalis* (Wake *et al*, 2000), the extract utilised in Chapter 10 was found to have comparatively low muscarinic, and extremely low nicotinic binding properties in human occipital cortex grey matter. The current chapter comprises an investigation of the cholinergic receptor binding properties of a selection of commercially available (and one locally grown) *M. officinalis* dried leaf samples. The aim of this study was to identify a *M. officinalis* that possesses properties that might, theoretically, confer it with the potential to beneficially modulate human cholinergic activity, and thereby enhance cognitive performance. The *in vitro* investigation of receptor binding properties followed the methodology of Wake *et al* (2000), and was undertaken at the MRC Centre for Development in Clinical Brain Aging, Newcastle General Hospital.

## Materials and Methods

### Plant Materials

A number of high quality commercial sources of *Melissa officinalis* were identified. A further sample (year 2000 crop) was obtained from the University of Newcastle's Moor Bank botanical gardens. In each case in excess of 1 kg of dried leaf was obtained. With the exception of Sample A (Moor Bank botanical garden) all leaves were harvested prior to flowering. All leaves were organically grown, with the exception of Sample B for which no information was available. Sourcing details are given below.

***Sample A.*** Moor Bank botanical garden – Year 2000 crop – harvested after flowering.

***Sample B*** Imported from Hungary, no other information available. Bought from Newgate herbs, Newcastle upon Tyne.

***Sample C.*** Organically grown leaf of Spanish origin, bought from Nealls Yard Remedies, Newcastle upon Tyne.

***Sample D.*** Organic herb of Spanish origin. Bought from the Organic Herb Trading Company, Milverton, Somerset, batch 8524.

***Sample E.*** Organic leaf of French origin. Bought from the Organic Herb Trading Company, Milverton, Somerset, batch 8531.

***Sample F.*** Organic wild Bulgarian herb. Bought from the Organic Herb Trading Company, Milverton, Somerset, batch 7249.

***Sample G.*** Organic herb from G. Baldwin, Walworth Rd. London. Polish origin.

***Sample H.*** Organic herb from G. Baldwin, Walworth Rd. London. Polish origin.

### Melissa extract preparation.

The *M. officinalis* samples were prepared for two separate analyses. In the first a crude ethanolic extract of the whole herb was assessed, in separate assays, for receptor binding to human cortex

nicotinic and muscarinic receptors. In the second a moderately polar fraction (containing terpenoids and phenolic materials) was isolated for muscarinic analysis, and a basic fraction (ionisable basic materials) was isolated for nicotinic analysis. The fractionation served two purposes. Firstly it was intended to confirm that any displacement properties of the crude extract were receptor specific, as opposed to being as a result of adherence by incidental plant components hindering the access of the radiolabels. Secondly, it served to highlight inhibitory or synergistic properties of the crude extract.

**Ethanolic extracts**

Ethanolic extracts were prepared by allowing coarsely pulverised 10 g portions of the various Melissa acquisitions to macerate for 7 days in 80% ethanol (aqueous) at 4 °C in darkness. The ethanolic solutions were then decanted from the extracted leaf residues, filtered through Whatman No. 1 filter papers and stored at -20°C in glass-stoppered conical flasks. For assay, the extracts were diluted 1:1 with 80% ethanol to bring the working solution to a concentration of 100 mg $ml^{-1}$ and to give an assay concentration of 10 mg $ml^{-1}$.

**Methanolic extracts**

Coarsely pulverised 20 g samples of the various *Melissa officinalis* acquisitions were allowed to macerate for 7 days in 200 ml aliquots of 100% methanol. The liquors were filtered from the leaf materials, and evaporated to $1/5^{th}$ volume on a Buchi rotary evaporator. The concentrated extracts were stored in their evaporating flasks at - 20°C. Recovered solvent plus fresh methanol to make up 200 ml solvent volumes were added to the herb residues which were then allowed to macerate a further 24 hours, after which the methanolic liquors were decanted from the leaf materials, filtered, added to the existing concentrates, and reduced in volume on the rotary evaporator.

The 24 hour extraction and evaporation cycles were carried out 5 times after the initial 7 day extraction. The final volumes of concentrated extracts were approximately 50 ml.

The methanolic *Melissa* extracts were fractionated using differences in pH and hydrophobicity. Each extract was separated into a moderately polar fraction (terpenoids and phenolic materials) for muscarinic analysis, and a basic fraction, (ionisable basic materials) for nicotinic analysis. Each fraction was concentrated to approximately 10 ml, transferred to weighed sample vials, evaporated to dryness on the rotary evaporator, weighed and stored at -20°C until required for assay. Fractionation yelds are shown in Table 1. For the assays, moderately polar fractions were prepared as 10 mg $ml^{-1}$ solutions in 80% ethanol and basic fractions as 2 mg $ml^{-1}$ solutions in the same solvent.

| Sample | Moderately polar fraction | | Basic fraction | |
|---|---|---|---|---|
| | *weight* | *% of dry weight of plant material* | *weight* | *% of dry weight of plant material* |
| **A** | 0.68 g | 3.4% | 0.0036 g | 0.018 % |
| **B** | 0.78 g | 3.9% | 0.0034 g | 0.017 % |
| **C** | 0.68 g | 3.4% | 0.004 g | 0.02 % |
| **D** | 0.503 g | 2.5% | 0.0091 g | 0.045 % |
| **E** | 0.96 g | 4.8% | 0.0041 g | 0.02 % |
| **F** | 1.07 g | 5.35% | 0.0043 g | 0.02 % |
| **G** | 1.41 g | 2.8% | 0.0065 g | 0.013 % |
| **H** | 1.07 g | 2.14% | 0.0063 g | 0.013 % |

Table 1. Yields by weight, and % of dry weight, of the moderately polar fraction, and basic fraction of the methanolic extract of the *M. officinalis* Samples.

**Preparation of brain membranes**

Human occipital cortex grey matter (obtained from the Newcastle General Hospital Brain Bank) was homogenised following the method described in detail by Wake *et al* (2000).

**Nicotinic and Muscarinic displacement assays**

The displacement of radio-labelled [$^3$H]-nicotine and [$^3$H]-scopolamine from brain homogenates by both the crude ethanolic extracts and methanolic fractions prepared from the *M. officinalis* samples, were undertaken using the method described by Wake *et al* (2000).

## Results

$IC_{50}$ values for displacement of radio-labelled [$^3$H]-nicotine and [$^3$H]-scopolamine from human occipital cortex tissue are shown in Table 2. Relative activity is ranked in order of effectiveness for both nicotine and scopolamine displacement, by both the whole ethanolic extract and the active fractions. In the case of the fractions, in order to make a valid comparison between samples, the rank reflects both displacement and the comparative yield of the fraction from the leaf for each sample.

| | Ethanol extract of whole herb | | | | Basic Fraction | | Moderately Polar Fraction | |
|---|---|---|---|---|---|---|---|---|
| Melissa Sample | $IC_{50}$ (mg ml) Nicotine displacement | Rank | $IC_{50}$ (mg ml) Scopolamine displacement | Rank | $IC_{50}$ (µg ml) nicotine displacement | Rank *Yield* / $IC_{50}$ | $IC_{50}$ (µg ml) Scopolamine displacement | Rank Yield / $IC_{50}$ |
| A | 0.18 | 1 | 3.47 | 4 | 8.6 | 5 | 162.9 | 5 |
| B | 0.23 | 2 | 3.08 | 3 | 7.9 (?) | 5 | 85.5 | 3 |
| C | 0.37 (?) | 3 | 2.69 | 2 | 7.5 | 4 | 143.4 | 4 |
| D | 0.40 | 6 | 3.5 | 6 | 4.2 | 1 | 134.1 | 7 |
| E | --- | --- | 1.46 | 1 | 4.0 | 2 | 102 | 2 |
| F | 1.12 | 4 | 3.48 | 5 | 10.0 | 5 | 102.6 | 1 |
| G | 3.16 | 5 | 3.98 | 7 | --- | --- | 160.8 | 8 |
| H | --- | --- | 4.31 | 8 | 8.0 | 3 | 104.6 | 6 |

Table 2 $IC_{50}$ values for displacement of radio-labelled [$^3$H]-nicotine and [$^3$H]-scopolamine from human occipital cortex tissue for the M. officinalis samples. Rank of omparative activity is also shown.

The primary indicator of relevant activity is displacement by the whole ethanolic extract, which reflects the co-ligands, synergists and binding inhibitors present in the dried leaf. However, displacement by the basic fraction and moderately polar fraction must also be substantial for any given sample of *M. officinalis*. This indicates that the demonstrated displacement is due to receptor binding rather than another property of the ethanolic extract (e.g. incidental binding by cell materials). A second indicator of true receptor binding is a sigmoidal dose response curve, with a lag phase, steeply ascending middle section and flattening at the upper limits as the last

receptor sites become occupied. In contrast to this a linear dose response curve suggests non-specific interactions or displacement of label bound to non-specific sites (Wake *et al,* 2000).

All of the assessed samples demonstrated significant levels of displacement of radioligand from muscarinic receptors. However samples E and H were excluded from further consideration on the basis of interference pattern nicotinic binding (indicated by an absence of an $IC_{50}$), and sample G was excluded on the basis of a similar pattern for nicotinic binding by the basic fraction. Of the remaining samples A, B and C all exhibited $IC_{50}$ scores suggesting relatively high displacement of both nicotine and scopolamine. Reference to their respective binding curves suggested, however, that sample C possessed an ethanol extract nicotinic dose response curve verging on interference, and it was excluded from further consideration.

The dose response curves for the remaining two samples are presented in Figure 1 and Figure 2..

Reference to the dose response curves for both extracts suggests that both possess a muscarinic displacement profile in keeping with genuine receptor binding. However, the results with regards nicotinic displacement are not as clear-cut. In the case of Sample A the whole ethanolic extract dose response curve lacks the typical lag phase. However, the basic fraction produces a sigmoidal dose response curve, suggesting that the extract possesses receptor binding properties that may be masked by other properties of the ethanolic extract at low doses. In the case of Sample B neither the ethanolic fraction, nor basic fraction produce the expected sigmoidal dose response curve.

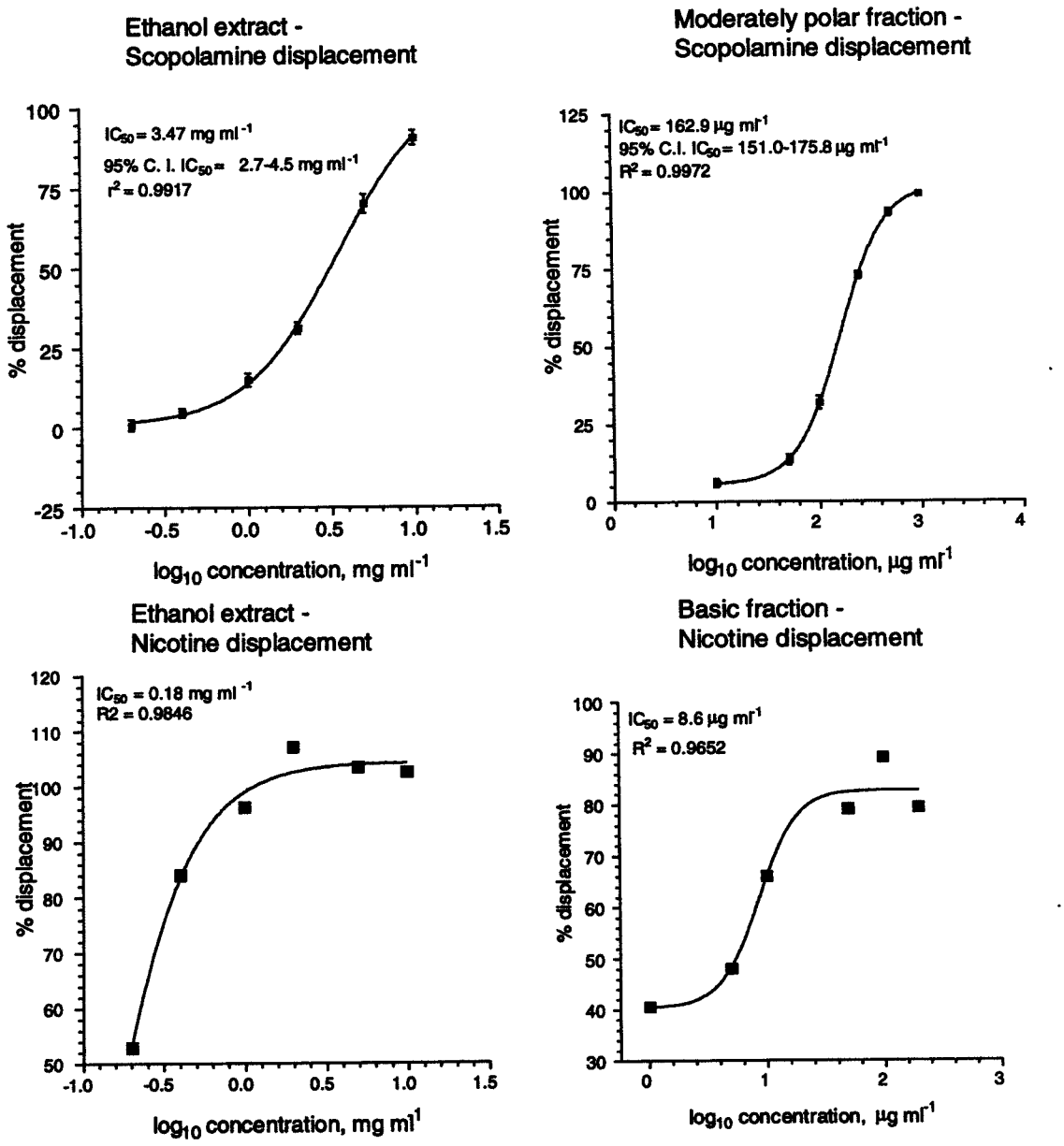


*Figure 1 showing dose response curves for the displacement from human occipital cortex tissue off radio-labelled [$^3$H]-nicotine by an ethanolic extract and basic fraction of melissa sample A, and [$^3$H]-scopolamine by an ethanolic extract and moderate polar fraction (terpenes) of melissa sample A.*

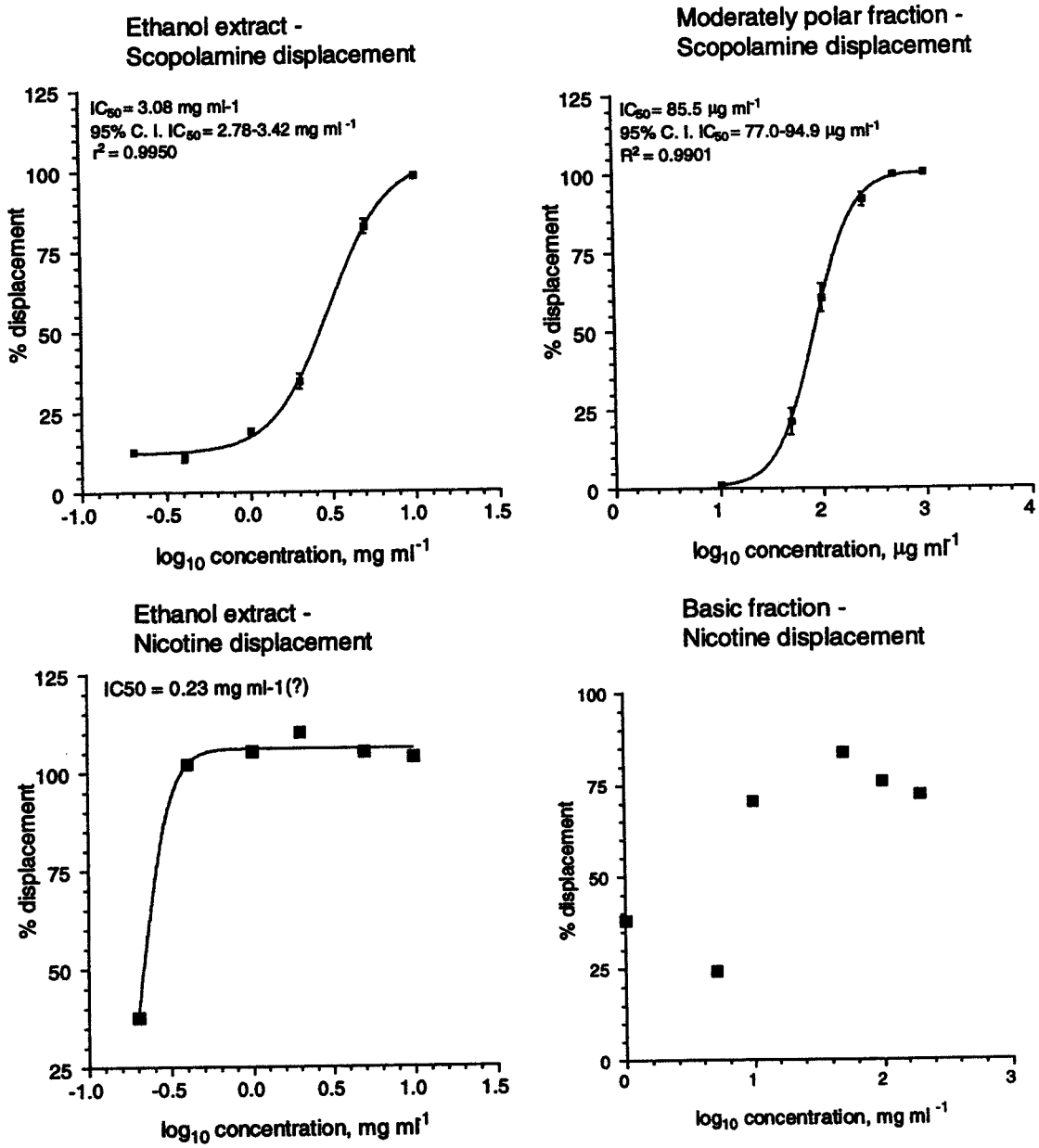


*Figure 2 showing dose response curves for the displacement from human occipital cortex tissue off radio-labelled [$^3$H]-nicotine by an ethanolic extract and basic fraction of melissa sample B, and [$^3$H]-scopolamine by an ethanolic extract and moderate polar fraction (terpenes) of melissa sample B.*

## Discussion

All of the samples of *M. officinalis* investigated demonstrated properties in keeping with substantial displacement of [$^3$H]-scopolamine from human occipital cortex muscarinic receptors. The case with regards displacement of [$^3$H]-nicotine was somewhat less clear-cut, with a number of samples failing to produce an overwhelmingly convincing profile of results. Two samples produced particularly promising $IC_{50}$ values for displacement of both radio-ligands by the ethanolic extract. In the case of both Sample A, procured from Moorbank Botanical Gardens, and Sample B, an imported Hungarian crop, the relevant $IC_{50}$ values were towards the upper end of values obtained for a range of *M. officinalis* accessions in a previous radioligand displacement study that used the same methodology as the current study (Wake *et al,* 2000). They were also, particularly in respect of nicotinic displacement, substantially in excess of those obtained for the melissa extract used in Chapter 10.

On closer examination Sample A was found to have a possible advantage, with sigmoidal dose response curves for the displacement both of scopolamine (by ethanol and the moderate polar fraction), and nicotine, most notably by the 'nicotinic' basic fraction. Sample B had a less convincing profile for its nicotinic dose response curve, both for the ethanolic extract and the basic fraction. One possible confounding factor with the latter sample is the lack of organic certification, and it is possible that the less than clear nicotinic results may be as a consequence of application of chemical products to the leaves.

In conclusion, bearing in mind the more promising pattern of results, and the known provenance of the Moorbank Botanical Gardens *M. officinalis*, Sample A has the most promising receptor binding properties of those samples of *M. officinalis* assessed here.

**Appendix II**

# VISUAL ANALOGUE SCALES

| | | |
|---|---|---|
| ALERT | _____ | DROWSY |
| CALM | _____ | EXCITED |
| STRONG | _____ | FEEBLE |
| MUZZY | _____ | CLEAR HEADED |
| WELL COORDINATED | _____ | CLUMSY |
| LETHARGIC | _____ | ENERGETIC |
| CONTENTED | _____ | DISCONTENTED |
| TROUBLED | _____ | TRANQUIL |
| MENTALLY SLOW | _____ | QUICK WITTED |
| TENSE | _____ | RELAXED |
| ATTENTIVE | _____ | DREAMY |
| INCOMPETENT | _____ | PROFICIENT |
| HAPPY | _____ | SAD |
| ANTAGONISTIC | _____ | FRIENDLY |
| INTERESTED | _____ | BORED |
| WITHDRAWN | _____ | SOCIABLE |